EVALUATION OF EFFECT OF PULSED ELECTROMAGNETIC FIELD THERAPY IN CHRONIC NON-HEALING DIABETIC FOOT ULCERS

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Government Madras Medical College and Hospital

CHENNAI –600003

THE TAMIL NADU DR. MGR MEDICAL UNIVERSITY

CHENNAI –600032

APRIL, 2016

CERTIFICATE

This is to certify that the dissertation entitled "**Evaluation of Effect of Pulsed electromagnetic field therapy in chronic non healing Diabetic foot ulcers"** by the candidate **Dr.AMARESWARI V.H.** for M.D Physiology is a bonafide record of the research done by her during the period of study(2013 – 2016) in the Institute of Physiology and Experimental Medicine, Madras Medical College, Chennai –600003.

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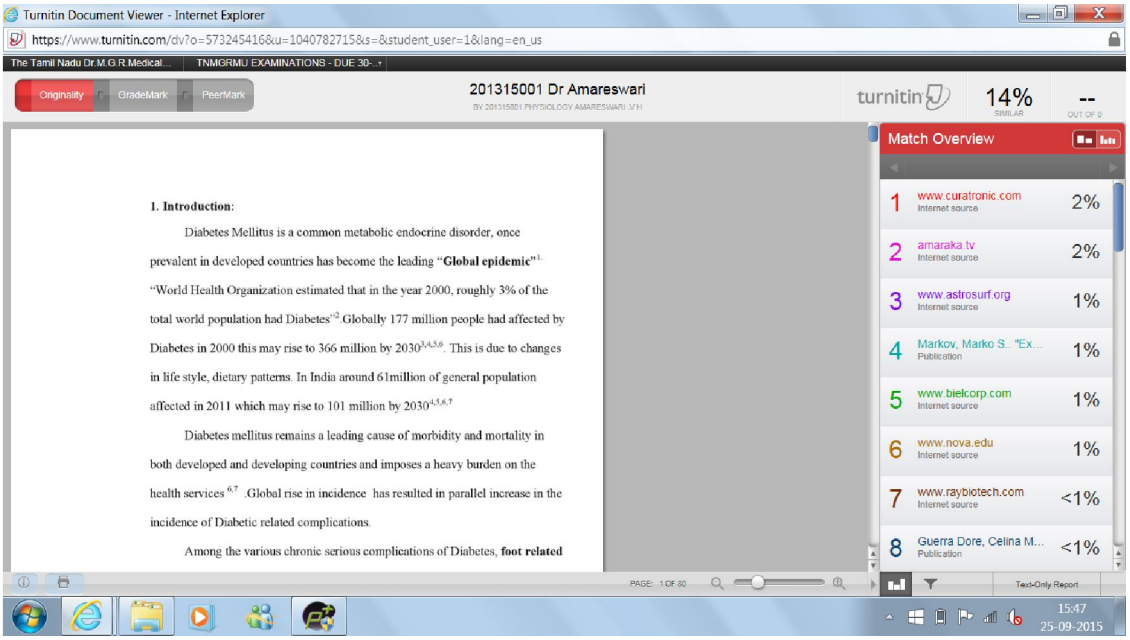
LIST OF ABBREVIATIONS

ROS	Reactive Oxygen Species
DPN	Diabetic Peripheral Neuropathy
HIF-1	Hypoxia inducible factor-1
PEMF	Pulsed Electro Magnetic Fields
VEGF	Vascular Endothelial Growth Factor
PGF	Placental Growth Factor
NO	Nitric Oxide
DAG	Di-Acyl Glycerol
PKC	Protein Kinase C
MMP	Matrix Metalloproteinases
DFU	Diabetic Foot Ulcer
HBOT	Hyper Baric Oxygen Therapy
DC	Direct Current
TENS	Trans cutaneous Electrical Nerve Stimulation
NOS	Nitric Oxide Synthase
CaM	Calmodulin
cGMP	Cyclic Guanosine Mono Phosphate
IL	Interleukin
TNF	Tumor Necrosis Factor
INF	Interferon
TGF-β	Transforming Growth Factor β
VPF	Vascular Permeability Factor
EPC	Endothelial Precursor Cells
NDS	Neuropathy Disability Score
PUSH	Pressure Ulcer Scale for healing
ELISA	Enzyme Linked Immuno Sorbent Assay
TMB	Tetra Methyl Benzidine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1. Introduction:
Diabetes Mellitus is a common metabolic endocrine disorder, once prevalent in developed countries has become the leading "Global epidemic"[1]. "World Health Organization estimated that in the year 2000, roughly 3% of the total world population had Diabetes"[2].Globally 177 million people had affected by Diabetes in 2000 this may rise to 366 million by 2030[3,4,5,6]. This is due to changes in life style, dietary patterns. In India around 61million of general population affected in 2011 which may rise to 101 million by 2030[4,5,6,7]
Diabetes mellitus remains a leading cause of morbidity and mortality in both developed and developing countries and imposes a heavy burden on the health services [6,7] .Global rise in incidence has resulted in parallel increase in the incidence of Diabetic related complications.
Among the various chronic serious complications of Diabetes, foot related
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ABSTRACT

EVALUATION OF EFFECT OF PULSED ELECTROMAGNETIC FIELD THERAPY IN NON HEALING CHRONIC DIABETIC FOOT ULCERS

Degree for which submitted: Doctor of Medicine (MD) in Physiology

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Chennai-600032

Year : 2014 – 2015

Back ground: Among the various chronic serious complications of Diabetes mellitus ***foot related complications*** top the list. The life time risk of developing foot ulcer is 25 % and the annual incidence is 2-3% and leads to devastating consequence limb amputation. Diabetic foot ulcers fail to heal and become chronic due to repetitive trauma, infection and ischemia that are characterized by inappropriate inflammation, disproportionate collagen synthesis, degradation and deficient growth factors such as VEGF. Conventional treatments fail to treat underlying pathogenesis. Pulsed Electromagnetic Field therapy is a new modality

which is found to interact at various steps in wound healing and up-regulates growth factors such as VEGF.

Aim & Objective:

To evaluate the effect of Pulsed Electro Magnetic Field therapy on chronic diabetic foot ulcers

Materials & Methods:

Thirty type 1 and 2 diabetic patients with chronic non healing diabetic foot ulcer of duration 4- 6weeks with Wagner's foot ulcer grade 1 and 2 were exposed to PEMF therapy for 45 min /day for 30 days. The wound size and dimensions, granulation tissue, exudates, serum VEGF levels were noted before and after the PEMF therapy.

Result:

The results were analyzed using paired t' test. The wound dimensions such as length, width, surface area, depth decreased significantly. Healthy granulation tissue and reduction in exudates were also observed. Significant elevation in Serum VEGF levels was also observed. All these results substantiate that PEMF therapy has enhanced wound healing.

Conclusion:

Decreased wound dimensions, formation of healthy granulation tissue, reduction in exudate and elevated serum VEGF levels indicate that PEMF enhance wound healing. No side effects were reported .Thus PEMF can be an effective and safe adjunct therapy for treating chronic non healing diabetic foot ulcers.

Keywords: Diabetic patients, PEMF, Wagner's grade, wound dimensions, granulation tissue, exudates, VEGF.

Introduction

Diabetes Mellitus is a common metabolic endocrine disorder, once prevalent in developed countries has become the leading "**Global epidemic"** (Apleqvist et al[1]). "World Health Organization estimated that in the year 2000, roughly 3% of the total world population had Diabetes" (Armstrong DG et al[2]).

Globally 177 million people had affected by Diabetes in 2000 this may rise to 366 million by 2030 (International Diabetes Federation; 2003[3], International diabetes federation Diabetes atlas 2011[4], International Diabetes Federation 2012[5], Wild S, Roglic G et al[6]).

This is due to changes in life style, dietary patterns. In India around 61million of general population affected in 2011 which may rise to 101 million by 2030 (International diabetes federation Diabetes atlas 2011 [4], International Diabetes Federation 2012 [5], Wild S, Roglic G et al[6], Alvin C et al[7]).

Diabetes mellitus remains a leading cause of morbidity and mortality in both developed and developing countries and imposes a heavy burden on the health services (Wild S, Roglic G et al[6], Alvin C et al[7]).

Global rise in incidence has resulted in parallel increase in the incidence of Diabetic related complications.

Among the various chronic serious complications of Diabetes, **foot related complications** top the list (Wild S, Roglic G[6], Vileikyte Let al[8], Reiber GE et al[9]).

The life time risk of developing foot ulcer in individuals with diabetes mellitus is 25 % and the annual incidence is 2-3% (Vileikyte L et al [8], Reiber GE et al[9]).

Development of foot ulcer changes the quality of life in patients leading to devastating consequences like limb amputation and remains the major risk factor for all non traumatic foot amputations (Wild S, Roglic Get al[6], Vileikyte L et al[8], Reiber GE et al [9], Leung PC et al[10]).

More than a million lower leg amputations are performed each year worldwide due to diabetes and every 30 second at least one lower limb is amputated (International Diabetes Federation 2005[11], Boulton, A. J et al [12]).

In India around 100,000 leg amputations are carried out per year (Contemporary management of the Diabetic foot[13]).

The 2005 international diabetic federation reports that 85% of diabetes-related lower extremity amputations are preceded by a foot ulcer (Larsson, J et al[14]). "Foot problems account for up to 15% of healthcare resources in developed countries and 40% in developing countries" (International diabetes federation Diabetes atlas 2011 [4], International Diabetes Federation 2012 [5])

These amputations are potentially preventable. It has been established that a case strategy that combines prevention, multidisciplinary treatment of foot ulcers, appropriate organization can lead to significant reductions in amputation rates up to 85% (Larsson, J et al[14]).

Risk factors:

Multiple contributory factors play a role in the pathogenesis of diabetic foot ulcers (Larsson, J et al[14]).

A triad of neuropathy, vascular and biomechanical changes in the foot are considered to be the predisposing factors for the development of ulcers. Peripheral neuropathy and vascular changes are the two major factors that head the list (Contemporary management of the Diabetic foot[13], Larsson, J et al[14]).

Neuropathy, vascular changes or a combination of both produce mechanical stress in the foot and precipitate ulcers (Larsson, J et al 14, Armstrong DG et al[15], Bowering CK et al[16]). Most of foot ulcers are of neuropathic in origin. Around 55 to 60% of diabetic foot ulcers are caused by neuropathy (Dyck PJ et al [17]).

Old age, duration of diabetes, male gender, alcohol intake, poor glycemic control and smoking are other contributory factors in the pathogenesis of foot ulcers (Bowering CK et al.[16], Dyck PJ et al[17]).

Pathogenesis:

Hyperglycemia induced metabolic abnormalities leads to **peripheral neuropathy** (Zochodone DW et al [18] Huijberts MS et al[19]) .Hyperglycemic status elevates the enzymes aldose reductase and sorbitol dehydrogenase of polyol pathway, which converts much of intracellular glucose to sorbitol, and fructose (Huijberts MS et al [19] Feldman ELet al [20]).

These sugars accumulate, decrease myoinositol synthesis in the neuron ,this is pre requisite for normal nerve conduction.

Reactive oxidative species (ROS) also accumulate and reduces nitric oxide synthesis which is a vasodilator. As a result the nerve cell undergoes severe oxidative stress, and an increased vasoconstriction resulting in ischemia finally leading to nerve injury and nerve death ensues (Feldman EL et al[20]).

Abnormal non enzymatic glycosylation of cell proteins takes place which cross link with collagen and extracellular matrix proteins and decrease synthesis of nitric oxide, produce abnormal endothelial function with alteration in extracellular matrix structure and integrity (Huijberts MS et al [19] Feldman EL et al [20]).

Diabetic Peripheral Neuropathy (DPN):

Affects motor, sensory and autonomic nervous systems (Zochodone DW et al[18], Feldman EL et al [20]).

Motor neuropathy:

Intrinsic foot muscles get atrophied in motor neuropathy with subsequent development of anatomical changes in the foot and deformities. The balance between flexion and extension movements is lost with development of high pressure points. The foot develops bony prominences that are abnormal. The superficial skin gradually breaks down and ulcer develops (Carine Hm, et al [21]).

Autonomic neuropathy:

Decreases sweat and sebaceous gland secretion. The overlying skin loses its moisture becomes dry and fissured. This increases susceptibility to cracks and thus infection (Carine Hm, et al [21]).

Sensory neuropathy:

Makes the patient insensitive to the triggering trauma and the injuries are left unnoticed. They are prone for repetitive trauma due to weight bearing and excessive pressure points during ambulation.

Peripheral vascular changes:

Is one more added contributory factor in the development of foot ulcer. Persistent hyperglycemic state leads to abnormal endothelial cell and smooth muscle function in peripheral arteries (Zochodone DW et al [18]).Decreased vasodilator Nitric oxide levels leads to vasoconstriction, increases the risk of plasma hypercoaguability, along with abnormal extracellular matrix of the vessel leads to narrowing of the arterial lumen (Carine Hm et al [21]).

The resultant ischemia ends in occlusive arterial disease in the lower limb with increased risk of ulcers (Paraskevas KI et al [22]).

Vascular changes leads to inadequate local blood flow which in turn favors infection and delay the wound healing. The above factors along with

biomechanical changes in the foot contribute to the development and chronic nature of the ulcer.

Diabetic Foot ulcers:

Halt at any stage of healing and remain in that stage for longer. The basic etiology prevents the anatomical and functional integrity back to normal unless interfered properly (Mostow EN et al[23], Nomikos I N et al[24]).

Persistent neutrophils and macrophages, release inflammatory cytokines and enzymes that damage cells (Snyder, Robert J et al[25]).Cytokines interfere with cell proliferation, wound closure and healing (Alleva, Renata et al[26]).keratinocytes fail to reepithelialize the wound and fibroblasts fail to produce adequate extracellular matrix proteins (Foy et al[27]).

Reactive Oxygen Species (ROS) and myeloperoxidase enzyme released and damage DNA, lipids proteins, extracellular matrix (ECM).

Growth factors formation is prevented or gets sequestered. Their normal metabolic functions are not carried out. Proteases from various cells and bacteria degrade them excessively (Crovetti et al[28])

Proteolytic enzymes like elastase (Schönfelder et al [29]) and **matrix metalloproteinase are higher** (Wysocki et al[30]).The released elastase inhibits proteases inhibitors (Edwards et al [31]) and leads to more inflammatory reaction. It damage tissue collagen, fibronectin, proteoglycans and growth factors.

Excess matrix metalloproteinase released by leukocytes breaks down ECM molecules and favour degradation while minimizing deposition with imbalance between production and degradation (Ravanti L et al[32], Vaalamo M et al[33]).

Reactive oxygen species are generated and inhibit normal angiogenesis. They affect HIF-1 (Hypoxia inducible factor-1) stability, limiting endothelial progenitor cells mobilization from the bone marrow into circulation and wounds thus inhibiting angiogenesis which is a major component in wound healing (Botusan IR et al[34]).

Disordered immune response thus prolongs the inflammation and breaks down the wound. Failure of reepithelialization makes the wound more prone to early infections and damage the tissues further leading to gangrene and limb amputation (Mustoe, Thomas et al[35]).

Foot infections are common and sometimes remain the immediate cause for amputation. Furthermore the infections are commonly ignored by many patients and report to the hospital only with gangrene of foot and sepsis. At this point it becomes challenge to treat them with conventional management.

Early diagnosis, prevention and management are essential to avoid this devastating complication.

The following strategies are implemented for prevention of amputation rates in diabetic foot ulcer foot syndrome.

1. Regular inspection of foot and foot wear

2. Preventive foot wears to high risk feet
3. Multidiscipline approach to the management foot ulcer
4. Early diagnosis of peripheral neuropathy and peripheral arterial disease
5. Continuous follow up of patients

Treatment:

Diabetic foot ulcers are conventionally treated by off loading and debridement (Armstrong DG, Lavery LA et al[36], Armstrong DG, Nguyen HC et al [37]).It is uncertain if these procedures can treat the cause of ulcers.

Other treatment modalities like hyperbaric oxygen therapy is in use but presently have conflicting data regarding the efficacy of this therapy(Faglia E et al [38]) and surgical therapies are also popular but costly.

Few new adjunctive therapies like use of colony stimulating growth factors are under investigation. Some of these studies revealed nil results in resolution of infection in diabetic wounds (Cruciani M et al[39])

Use of **non invasive methods** like the electro physical modalities is under trial which have been used for many years. There are many studies that these can act as adjuvant therapy in promoting early healing in chronic non healing diabetic foot ulcers. Ultra sound therapy, laser therapy and electromagnetic field therapies are in wide use.

Pulsed electromagnetic field (PEMF) therapy:

PEMF is becoming popular as an adjunct therapy for soft tissue healing. It is specifically designed such that it emits low frequency and low intensity electromagnetic fields to the tissues concerned and thus facilitates healing. It displays frequencies at the lower end of the electromagnetic spectrum.

From many studies it is evident that electromagnetic fields when applied externally to soft tissues either interacts directly with the local wound currents or with cellular transduction signaling pathways (Lee RC et al [40]) and thus play a role in re stimulation of wound that has retarded or arrested.

In the healing of soft tissue wounds the underlying concept in application of electromagnetic field therapy is that the induced electric fields have beneficial action at the level of cellular processes and functions involved in the wound repair.

. It is a safe and an easy procedure and feasible. Because of its non contact delivery to the tissues the chance of infection is prevented.

The electromagnetic field therapy has influence at various levels of wound healing such as (Lee RC et al [40], Gentzkow GD et al[41])

1. Chemotaxis of inflammatory cells such as neutrophils and macrophages
2. Upregulation of various growth factor receptors
3. Proliferation of fibroblast and granulation tissue
4. Epidermal cell migration
5. Blood flow increase and edema reduction

It has been used for promoting tissue healing over the last few decades (Ieran et al [42]).

PEMF promotes growth factors activity and increase their levels in the blood, and found to increase the rate of nerve regeneration in animal and vitro studies (Ito H. & Bassett C.A. et al[43]).

In human studies it is found to reduce pain, improves vibration sense, muscle activities, spinal cord motor excitability and restores nerve conductive dysfunction that is caused by diabetic polyneuropathy which is a major risk factor for developing diabetic foot ulcers (Musaev A.Vet al[44]).It Increases blood flow to the nerves and combat tissue hypoxia with no side effects reported (Webb, C. Y et al [45]).

As a result it is evident that the pulsed electromagnetic fields act as a potential, safe and an effective conservative management in promoting diabetic ulcer in clinical practice.

Vascular Endothelial Growth Factor (VEGF):

It is a signaling protein which induces vasculogenesis and angiogenesis. It was first isolated from ascitic proteins in 1983 and found to induce angiogenesis in cell culture. It was previously called as vascular permeability factor (Senger DR et al [46]).

The VEGF family includes five subfamilies: VEGF-A, VEGF-B, VEGF-C and VEGF-D and placental growth factor (PGF).

VEGF-A is commonly called as vascular endothelial growth factor (Robinson CJ et al[47]).Its main function is to improve blood supply and oxygenation to the tissues. It also stimulates endothelial cell mitogenesis and cell migration, increases matrix metalloproteinase activity, chemotactic for macrophages and produces vasodilatation by releasing Nitric Oxide.

Hypoxic cells stimulates the release of VEGF –A into circulation by a transcription factor known as hypoxia inducible factor .VEGF bind to VEGF receptors on endothelial cells and leads to angiogenesis (Holmes et al[48]),which is a vital component of wound repair (Brem H et al [49])

Diabetic foot ulcers and VEGF:

Though various conditions favour expression of vascular endothelial growth factor in diabetic wounds it is actually defective.

Induced full thickness excision wounds in animals showed initial increase but subsequent undetectable VEGF levels (Frank S et al[50]).Diabetic mice on induction of wounds with streptozotocin has showed defective synthesis of VEGF and other growth factors (Shukla A et al [51] ,Rivard A et al[52]).

As diabetic foot ulcers has multiple physiological impairments to healing, including impaired innervations (Gibran NS et al[53]), impaired cellular migration (Brem H, Stojadinovic O et al [54])and inadequate angiogenesis(Cho CH et al [55])it is vital to introduce a therapy that interacts with all these cascades of events. It is hypothesized that electromagnetic stimulation influences the migratory,

proliferative and synthetic functions of fibroblasts and also result in increased expression of growth factors (Lee RC et al [40],Webb, C.Y et al[45] Weiss DS et al [56]).

VEGF action in turn mediated by nitric oxide which leads to deposition of collagen reverts back endothelial dysfunction, improves nerve conduction and oxygen supply to the tissues (Witte MB et al [57]).

It was suggested that PEMF acts on Ca2+ calmodulin signaling mechanism leading to nitric oxide (NO) production (Markov MS, Wang S et al [58,] Markov MS, Pilla AA et al, 1994 [59] ,Markov MS, Pilla AA et al1997 [60])and growth factor cascades involved in tissue healing.

It is evident that PEMF works through NO cascade. This study focused on nature of healing in chronic diabetic foot ulcers following exposure to pulsed electromagnetic fields therapy with subsequent up regulation of vascular endothelial growth factor.

REVIEW OF LITERATURE

Review of literature

2.1.1. Diabetes mellitus:

Diabetes mellitus is characterized by hyperglycemia which occur either due to absolute deficiency of insulin or due to failure of insulin action on insulin receptors in the tissues.

In the absence of insulin carbohydrate, protein and fat metabolic abnormalities result in the target tissues. Despite surrounded by high glucose the tissues cannot utilize glucose either due to insulin deficiency or due to insulin resistance.

2.1.2. Classification:

Old classification of diabetes is revised and new classification given by American Diabetes Association expert committee in 2003 and Diabetes is classified into two broad etiopathogenetic categories (American diabetes association 2012 et al[61])

Type 1 Diabetes mellitus (Insulin dependent diabetes or Juvenile diabetes):

It is associated with profound and absolute insulin deficiency due to β cell destruction or autoimmune disease of pancreas and requires insulin replacement therapy. It accounts for only 5-10% of diabetic population.

Type 2 Diabetes mellitus (Non Insulin Dependent Diabetes, Adult onset diabetes):

The patients have a normal plasma insulin level and usually do not require insulin replacement therapy. They have relative insulin deficiency with insulin resistance. It accounts for about 90-95 % of diabetic population.

Among the two, type II diabetes is more prevalent in older people above 60yrs and is mostly undiagnosed until the late complications ensue due to lack of symptoms (Harris M.I.et al [62], Genuth S et al[63]).

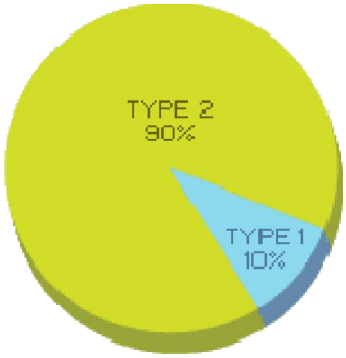


Fig-1. Prevalence of Types of Diabetes Mellitus

2.1.3. Complications:

Uncontrolled diabetes may result in acute life threatening complications like "Diabetic ketoacidosis or the nonketotic hyperosmolar syndrome" (Genuth S et al[63]).

Diabetes with associated Long-term complications includes:

1. Diabetic peripheral neuropathy and associated complications
2. Diabetic nephropathy with kidney failure

3. Diabetic retinopathy and associated complications

4. Autonomic neuropathy with associated Cardiovascular, gastrointestinal, sexual dysfunction and genitourinary complications (Genuth S et al[63]).

Atherosclerosis, peripheral arterial diaease , cardiovascular, and cerebrovascular diseases are other common complications that occurs in diabetic patients. Metabolic X syndrome which is characterized by hypertension and lipoprotein metabolism irregularities are commonly seen in diabetes patients (American diabetes association 2012 et al[61])

Diabetic foot ulcer is one of the common complications. It is the major component of the diabetic foot syndrome. It occurs in 15 % of all patients with diabetes.84 % of all diabetic –related lower –leg amputations are preceded by a diabetic foot ulcer (Brem, H et al[64]).

2.1.4. Diabetic foot ulcer:

An ulcer is defined as the **"discontinuity in the epithelium"** and a breach in the skin of foot including toes, heel, dorsum and the plantar aspect in diabetic patients is called as diabetic foot ulcer (Centers for Disease Control and Prevention 2003 [65])

2.1.5. Risk factors:

- Diabetic neuropathy
- Diabetic nephropathy
- Peripheral vascular disease
- Ulcers or amputations of previous history
- Smoking
- Uncontrolled Diabetes" (Scott, G et al[66])

2.1.6. Pathophysiology:

Diabetic peripheral neuropathy, peripheral vascular disease and accompanied biomechanical changes in the foot are the underlying major risk factors in diabetics that lead to foot ulcers (Mehmood K, Akhtar T et al[67])

There exists an interaction at various levels in the pathogenesis of neuropathy and vascular disease. The common basic pathogenesis is seen as follows.

1. Polyol or Sorbitol pathway:

Hyperglycemia increases the enzymes of sorbitol pathway (aldose reductase and sorbitol dehydrogenase).This reaction converts intracellular glucose into sorbitol and fructose. Elevated aldose sugars form advanced glycosylation end products which in turn leads to non enzymatic glycosylation of intra or extracellular proteins.

AGEs cross-link with proteins like collagen, extracellular matrix proteins, slow down their turnover rate, and alter extracellular matrix composition and structure (Reiber, G.E. et al[68]) and this collagen becomes inflexible, easily break down.

2. DAG/PKC Pathway:

Increased triose phosphate metabolites leads to increased second messenger diacylglycerol thus activates protein kinase C (PKC) which alters transcription of genes for fibronectin, type IV collagen, contractile proteins, and extracellular matrix proteins in endothelial cells and neurons.

Arachidonate in DAG is a substrate for synthesis of ecosanoids (prostaglandins,prostacyclins,thromoxanes and leukotriens) which are potent vasoconstrictors.

3. Oxidative Stress:

Decreased NADPH/NADP leads to alteration in redox state and the ability to deal with oxidative stress is decreased.

Increased aldose sugars alter redox potential, increases cellular osmolality.

Hyperglycemia induces mitochondrial dysfunction. Glucose and other metabolites undergo auto oxidation. Advanced glycation end products generate reactive oxygen species and increase oxidative stress. Resulting Oxidative stress leads to endothelial dysfunction and diminish nitric oxide synthesis and release.

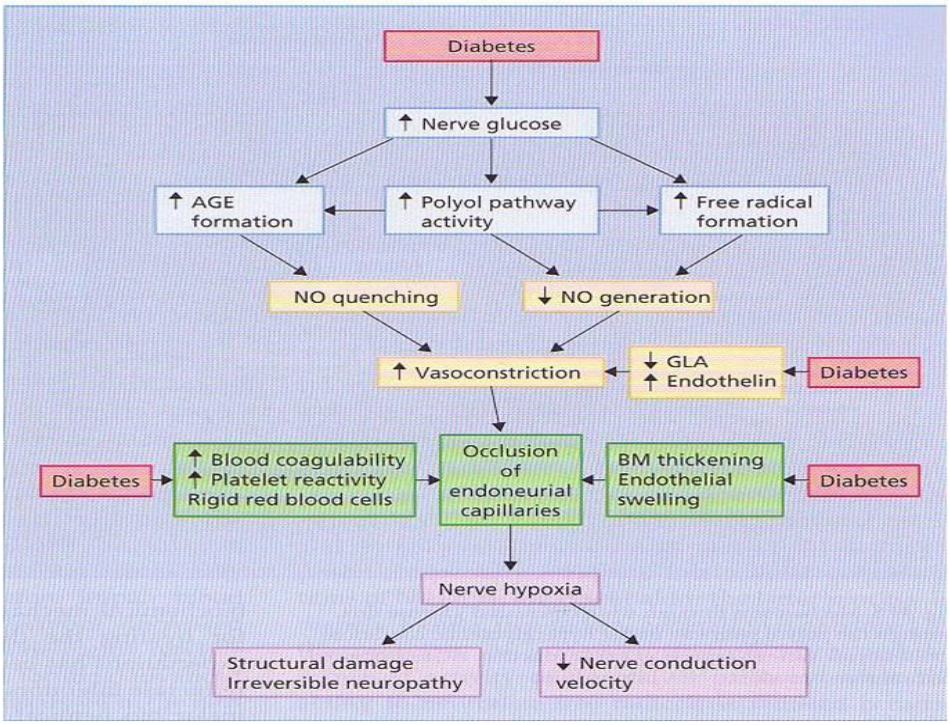


Fig 2: PATHOGENESIS OF NEUROPATHY

Nitric oxide synthase inhibitors get accumulated by polyyol flux pathway and results in reduced nitric oxide synthesis (Goldin, A et al[69]).Nitric Oxide is important for normal blood flow and it maintains the diameter of the blood vessel(Obayashi, K et al[70], Duda, D et al [71,]Dinh T et al[72]).Therefore the absence of NO results in vasoconstriction which leads to occlusion of endoneural capillaries and nerve hypoxia ensues.

Diabetic poly neuropathy: Microangiopathy of the vaso nervosum, structural damage, axonal atrophy and axonal loss results in diabetic poly neuropathy(Brownlee M et al[73])Accumulation of sorbitol and fructose in Schwann cells lead to decreased nerve myoinositol, reduced Na-K ATPase activity,

impaired axonal transport, abnormal action potential propagation and breakdown of nerve structure. All the three motor, sensory and autonomic neurons are affected.

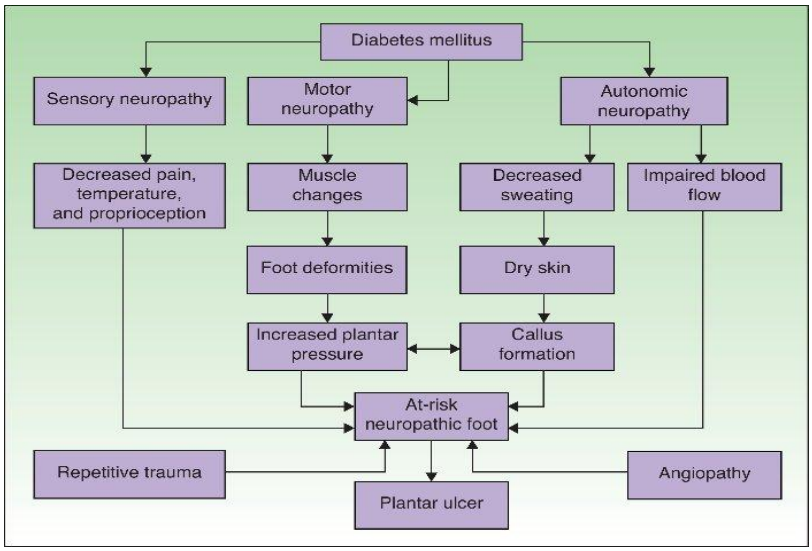


Fig.3.Pathogenesis of foot ulcer

Sensory neuropathy:

It is the key element in the development of ulcers (Reiber, G.E. et al[68]). Impaired sensation prevents withdrawl of foot from any painful stimuli and thus easily get injured while walking with bare footed (Boulton, A Jet al[74] ,Bus, S.A et al[75]).

Motor neuropathy:

Damage to motor neurons results in weakness, wasting and atrophy of intrinsic and interosseous muscles in the foot. A functional and structural change

in the foot ensues, with resultant loss of balanced gait and deformities in the affected foot (Feldman EL et al[20])

Various structural and biomechanical abnormalities are (Fryckberger RG et al[76], Lawrence L, et al[77])

1. Clawing of toes

2. Cavus Foot deformity:

The normal foot shape is convex as the longitudinal medial arch extends between first metatorsophalyngeal joint head and the calcaneus bone. Diabetics have high arch with resultant irregular distribution of pressure loads while walking and which leads to callosities in the foot.

3. Equinus Deformity:

Achilles tendon shortening with collapse of the plantar fascia facilitates abduction or adduction equinus deformity.

4. Rigid 1st toe:

The first MTP joint harden and fail to dorsiflex. The plantar surface undergoes excessive weight forces and results in callus.

5. Stiffness of the joint:

Abnormal glycosylated collagen in the joints leads to restricted mobility with periarticular tissues thickening. Foot abnormalities develop. Plantar flexion is limited due to excessive pressure points and equinus foot deformity. Talar, subtalar, MTPJoints are involved.

The normal feet while making contact with the ground absorb any shocks that develop on walking. This is impaired in diabetic patients due to the above mentioned changes .Bony prominences develop underneath the foot pushing the fibro fatty shock-absorbing tissues forward, exposing the condyles of the metatarsal heads (Contemporary management of the diabetic foot[78], Lyons et al[79])

Plantar pressure distributing capacity is reduced and favours high foot pressures in diabetics.

Normal foot arch support is lost in these patients during weight bearing. Soft tissue thickening at metatarsal heads contributes to high pressure (Cavanagh, P. R. et al[80]) this makes these ulcers to be present more commonly underneath metatarsal heads (Gefen, A. et al[81]).

A normal foot usually prevents the development of ulcers by redistributing the load equally over the entire surface -the forefoot, middle foot and rear foot". In diabetics, because of architectural changes in the foot this capacity is reduced.

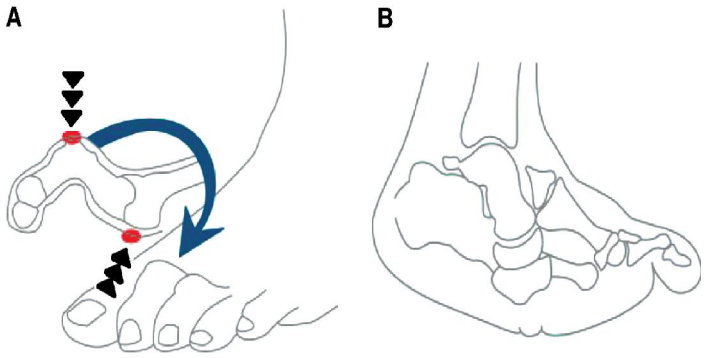


Fig-4. Foot deformities

Picture-A: shows clawing in the toe with plantar and dorsal aspects receiving more pressure.

Picture B: shows Charcot arthropathy in which the plantar aspect of mid foot received more pressure.

6. Nail Deformity:

In grown nails are commonly seen in diabetics due to nail thickening , atrophy of the nail plate with convex shaped deformity that give pressure on the ridge tissues. Due to inflammation and pressure the nail flange forms a callus and susceptible to trauma, infection and ulceration (Fryckberger RG et al [76] ,Lawrence L, et al[77]).

Plantar foot pad integrity:

Plantar foot pad located under the ball of the foot, beneath metatarsal heads, calcaneous or heel and provides in built cushion mechanism. Normally metatarsal

heads and the calcaneous bear the peak load on weight bearing and hence benefit from the most of the protective, cushioning provided by the plantar fat pad ((Gefen, A. et al[81]).Where there is clawing or retraction of the lesser digits, the plantar fat pad under the metatarsal heads migrates and is displaced anteriorly leading to ulcer.

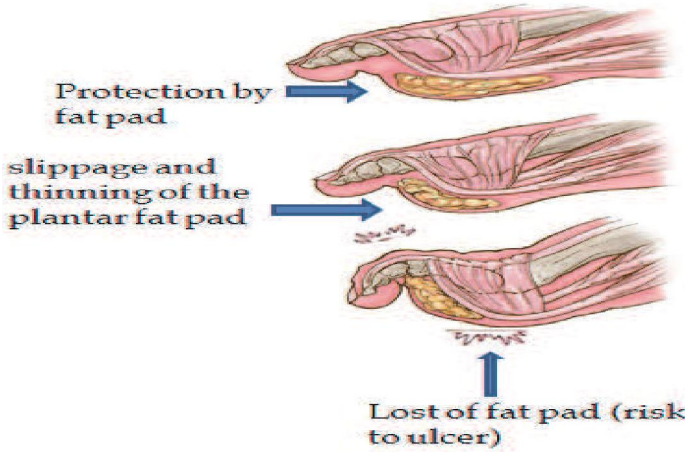


Fig-5. Foot fat pad integrity

Autonomic neuropathy:

Leads to impaired blood flow, diminished sweat, loss of overlying skin moisture finally ends in dry, cracked or fissured skin with fractures and callosities around the foot injury.

Callus or Callosities:

These are focal areas of increased pressure at weight bearing areas and acts as a foreign body exerting a concentrated pressure on underlying tissues thus are the potential sites for the ulcers to develop.

There is strong relationship between callus and other hyperkeratotic skin lesions and diabetic foot ulcers (Fernando. D. J et al[82])

They are more commonly seen in the rim of heel, plantar medial and first MTP joint and there is increased susceptibility to tears with subsequent development of infection and cellulitis (Brem, H., Sheehan, P et al[83]).

Peripheral Vascular Disease:

Persistent hyperglycemia results in abnormal Endothelial cell function and smooth cell abnormalities in the vessels(Zochodone DW et al[18])

Endothelium-derived vasodilators (NO) decreased leading to vasoconstriction. Diabetics have hypercoagulability status due to increased thromoxane A_2.

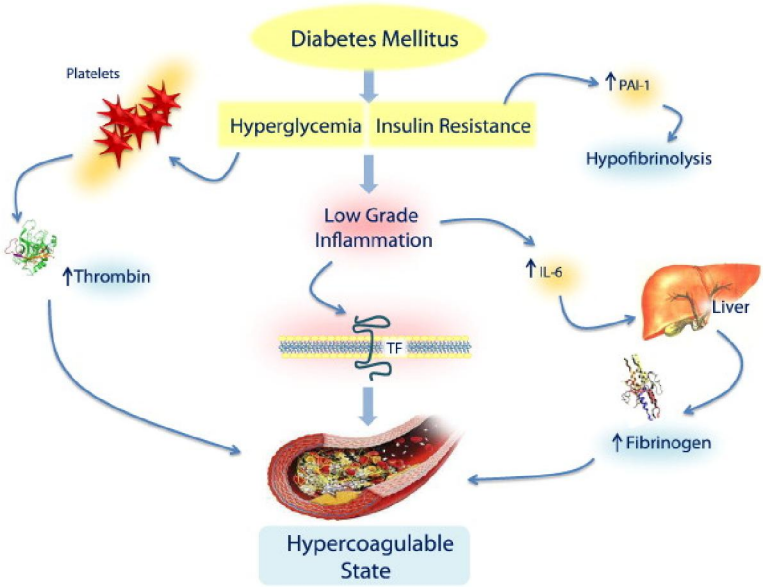


Fig-6. Hypercoagulable state in diabetes mellitus

Macro vascular and micro vascular changes lead to decreased capillary blood flow in the nutrient vessel and results in ischemia. Microvasular changes include capillary size reduction, basement membrane thickening and AV shunting[61] with reduced capillary recruitment.

Inadequate perfusion in the tissues leads to defective auto regulation of the vessels permeability changes and chemotaxis of leucocytes (Dinh T et al[72])

Macrovascular changes include atherosclerosis, occlusive narrowing of the vessel with resultant reduction in nutrient blood flow and ischemia. There is risk of stenosis in the arterial lumen due to changes in the vascular extracellular matrix (Fryckberger RG et al [76]).Tibial and peroneal arteries of the calf are commonly affected with subsequent occlusion.

The sympathetic blood flow which controls arteriovenous shunts is reduced. Decreased arteriovenous pressure over capillaries reduces flow in the nutritive vessels and leads to impaired skin capillary circulation in the toes.

Thus skin integrity is lost, becomes dry and portal of entry to bacteria. Impaired micro and macro vascular circulation lowers oxygenation in the wound (Nazimek-siewniak, B et al[84]) and contributes to nerve hypoxia (Malik, R. A. et al[85]).

In addition to major contribution of neuropathy and vascular disease difficulty in wound healing is an important and independent component.

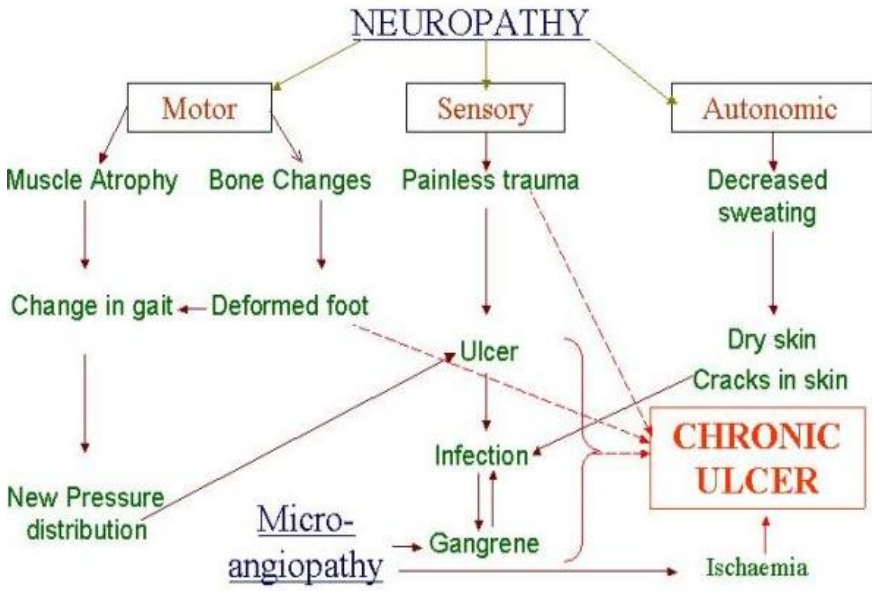


Fig-7.Pathogenesis of Chronic Ulcer

2.1.7. Diabetic ulcer a chronic ulcer:

Acute wounds normally heal by stage of inflammation, proliferation and remodeling (Shai. A. & Maibach. H. I. et al[86]).Blood vessels constrict to arrest bleeding and release inflammatory cells that initiate inflammation followed by vasodilatation which increases vascular permeability, recruits neutrophils and macrophages to the wound site.

These cells fight against pathogenic organisms, secrete various growth factors and cytokines .The released growth factors and cytokines recruit fibroblasts, epithelial cells and keratinocytes to the wound area. Angiogenesis occurs by endothelial proliferation.

Wound heals by formation of granulation tissue, collagen deposition and epithelialization.There is sequential and timely healing with overall restoration of

functional and anatomical integrity. Collagen type 3 is converted into more stable collagen type 1 which is arranged in orderly manner and results in scar formation. Tensile strength increased with precise balance between collagen deposition and degradation (Shai. A. & Maibach. H. I. et al[86]).

Diabetic ulcer is characterized by **Retarded Wound Healing**

Inadequate blood supply to the tissues with resultant hypoxia interferes with proper wound healing and converts it into a chronic ulcer.

Muscle atrophy, bone defects, deformed foot and gait changes that leads to new pressure areas with repetitive trauma and recurrent infection leads to development of chronic ulcers. They become refractory to heeling.

A chronic ulcer is the one that takes more than three months to heel. Less vascular endothelial growth factor (VEGF) is released from macrophages. Matrix metalloproteases (MMPs) are excessively activated and MMP2, MMP9 over expression (Wysocki et al [30]). Metalloproteinase-2 inhibitors in the tissues are very low thus extracellular matrix and growth factors are degraded excessively.

MMPs also generate antiangiogenic factors with reduced vascularity and angiogenesis which is characteristic of DFUs.

2.1.8. Diabetic foot infections:

Hyperglycemia act as culture media and increases the virulence of microorganisms. Diabetic foot infections present as simple as paronychia, to severe infections like onychomycosis, cellulitis etc.

2.1.9. Classification of diabetic ulcers:

Wagner Ulcer Classification system is based on depth of the wound and the necrotic tissue extent (Wagner FW Jr et al[86]).

Wagner ulcer classification system	
GRADE	LESION
1	Superficial diabetic ulcer
2	Ulcer extension involving ligament, tendon, joint capsule, or fascia with no abscess or osteomyelitis
3	Deep ulcer with abscess or osteomyelitis
4	Gangrene to portion of forefoot
5	Extensive gangrene of foot

The University of Texas system is based on depth of the ulcer, presence or absence of infection and ischemia (Oyibo SO, Jude EBet al[87]).

University of texas wound classification system	
STAGES	**DESCRIPTION**
Stage A	No infection or ischemia
Stage B	Infection present
Stage C	Ischemia present
Stage D	Infection and ischemia present

Grading	Description
Grade 0	Epithelialized wound
Grade 1	Superficial wound
Grade 2	Wound penetrates to tendon or capsule
Grade 3	Wound penetrates to bone or joint

Higher grade and stages fail to heal unless intervened like vascular repair or amputation (Frykberg RG et al[88])

2.1.10. Treatment of diabetic Ulcer:

Reverting back the blood sugar is the basis in the management thus preventing the related complications. The treatment depends upon the severity of the ulcer and ischemic status. The primary interventions to be carried are (Aguilar F, Steed, D. L. et al[89,90]).

1. Debridement of necrotic tissue and surrounding edges
2. Reduction in off loading and Foot rest ---orthotics
3. Antibiotics to get rid of infection
4. Wound care.

1. Debridement:

Necrotic and non viable tissue with thick callus is removed without fail otherwise this tissue fails to regenerate and interfere with healthy tissue formation.

Superficial wounds without bone or tendon involvement treated with enzymatic debridement or autolytic debridement. Early removal of devitalized tissue and enhancement in healing process are the advantages.

Autolytic debridement occurs naturally and wound exudates contain leucocytes and autolytic enzymes. Though slow, this process removes devitalized tissue from the wounds.

Debridement begins healing process by activating platelets, keratinocytes migration and growth factors release (D. L. et al[90]).

2. Off-loading:

Off-loading is an essential component of healing of foot ulcers as they are common to occur in high pressure areas of foot with sensory loss. There is retardation of healing and increased risk for complications if pressure is not reduced. Total contact casting is an effective off-loading technique for treatment of neuropathic wounds (D. L. et al[90]).

In total contact cast, the cast is minimally padded and molded carefully to the shape of the foot. These special casts redistribute weight off the ulcer site and allow patients to walk while the ulcer heals (Hartsell, H. D.et al[91])

Though successful this method is not advisable to all diabetic foot ulcers, also it requires experienced, specially trained technicians because inappropriate application of the cast may worsens the ulcer and infection.

3. Treatment of Infection:

Cellulitis,osteomyelitis and other deep tissue infections complicate diabetic ulcers if not treated ends in septicemia and death.

Wound culture for bacteriological diagnosis is to be done, topical antibiotics to be started empirically and x-rays to be taken to rule out the possibility of abscess or osteomyelitis. Parenteral antibiotics are used for infections in deep soft tissue infections, cellulitis and those with excess drainage.

4. Wound care:

Clean dressing with proper humidity and environment lead to recruitment of epithelial cells and growth factors and protects from dehydration and death. It hastens angiogenesis.

Newer Interventions:

1. Biological Therapy or cell therapy:

Diabetic foot ulcers are characterized by defective angiogenesis and growth factors. Cell therapy or biological therapy by releasing growth factors by increasing cytokines and extracellular matrix proteins and by promoting angiogenesis can be treatment option in some cases.

It has consists of biological layers of which an allogenic human keratinocytes on overlying allogenic human fibroblast layer suspended within a collagen matrix (Veves, A et al [92]).

For those ulcers with resistant to standard therapy this treatment has proven effective. Collagen, growth factors and matrix proteins which favour wound healing and epithelialization of the tissue synthesized by fibroblasts (Gath, H. J. et al[93]).The substances secreted by keratinocytes stimulate target genes, which control various cellular activation cycles that are responsible for the wound healing process. Though easy to apply as outpatient, inpatient and nursing home setups, they need several applications due to lesser half life of just 6 weeks.

2. Growth Factors:

By recombinant DNA technology synthetic growth factors are generated. They initiate wound repair by various cellular mechanisms like proliferation of and chemo taxis of the cells, new vessel formation, growth factors and other protein expression, and enzymatic production. They act within the wound and send signals to the target cells (Brem, H., Young, J et al[94]).

They can be applied topically and accelerate healing of the wounds, stimulates granulation tissue and enhance epithelialization (Steed, D. L. et al[90])

Usually they are much effective in healing if used as single or isolated forms as they influence different types of cells as platelet derived growth factor.

Recombinant human PDGF-BB is one of the best examples which have similar biological activity to that of natural PDGF. It promotes wound repair process by the recruitment and proliferation of cells (Embil, J. M. et al[95]).It is

found to be safe and pharmacologically proven active treatment for chronic lower extremity diabetic ulcers.

They are applied as a thin layer to the wound with a tongue depressor and covering it with a moistened saline gauze dressing and then rinsed off gently after 12 hours with changing the dressing. The procedure is repeated every 12-hours (Milington J.T. et al [96]).But further trials are needed to estimate the required concentration for optimal wound healing.

3. Negative Pressure Wound Therapy

This is a recent technique which utilizes a vacuum- assisted closure device refractory ulcers, unsatisfactorily healed ulcers, complex ulcers, and recurrent post operative or post amputation ulcers. It is found to result in rapid healing when compared to standard treatment (Armstrong, D. G.et al[97]).Ambulating patients wear these units around their waist and free movements are allowed to perform day to day activities.

4. Reconstructive mode of therapies:

Plastic surgeries are of use if the above explained approaches failed to reduce in the ulcer area more than 10 percent even after a 2 months period.

They high are presented with complications such as osteomyelitis and required revascularization before reconstruction in 52% and 42 percent of the patients respectively (Attinger, C. E. et al[98]).

5. Gene therapy:

This is an expensive procedure and it focuses on growth factor production as well as inhibits various proteases that inhibit growth factors.

Education of every professional who treat and the patient is utmost important thing to prevent and detect any complications of diabetes mellitus. "Updated medical information needs to cover all news about nutrition, hypertension, obesity and dyslipidemia (metabolic syndrome)".

Ulcer recurrence:

Previous ulcer or amputation usually associated with recurrent foot ulcers and about "60% of diabetic patients with a history of foot ulcer may develop another ulcer within a year of healing" ulcers (Luca DP et al[99]).The persistence of risk factors, fragile skin and soft tissues breaks easily at healed site are the reasons. Residual deformities formed at the areas of previous amputation area increase plantar pressure and leads to ulcer. Any previous surgery alters biomechanics of the foot results in gait abnormalities and loss of balance. New ulcers develop in the future at the high pressure areas.

Failure of surgical and non surgical treatment will end up in amputations. Even with best available treatment the outcome is unsatisfactory.

Many adjunctive therapies are also popular, among them **Hyper Baric Oxygen Therapy** most commonly used. Though earlier studies have showed a

beneficial effect of HBOT universal success is not reported (Kalani M et al [100]).It is also associated with complications like middle ear barotrauma and sinus pain.

Therefore a search of effective and new method to treat diabetic foot ulcers is needed. The use of physical modality is an alternative treatment approach for promoting healing in diabetic foot ulcers.

2.2.1. Electro physical therapy in diabetic foot ulcers:

Electro physical therapy consists of a variety of physical modalities ranging from electrical stimulation to the use of sound waves like ultra sound, laser to electromagnetic energy (Pope, G. D. et al [101]) .These modalities have been used to promote repair wounds, and fibroblast activity enhanced (Webb & Dyson, M.et al[102]) and angiogenesis (Goldman, R.et al[103]).There are many ongoing clinical studies for the use of electro physical modalities in diabetic wounds. Many studies were done on animal and in vitro studies. But it is uncertain whether these treatments have effects in diabetic ulcers (Gal, P.et al[104]).

2.2. 2.Pulsed electromagnetic field therapy in diabetic foot ulcers:

Use of magnetic fields as therapy in disease conditions has a long history. First scientific evidence is given in the book " **De Magnete**" written by William Gilbert during 1600 who was a personnel physician of the English Queen(Gilbert, W, et al [105]).After second world war II, Japan introduced contemporary

management of various diseases with magnetic and electromagnetic fields which is moved to Europe.

Todorov wrote the first book on electromagnetic therapy and its applications in various conditions (Todorov, N.et al[106]).In seventies "C.A.L.Bassett" and team proposed a modern approach using low frequency signal to treat delayed bone fractures (Basset ,C.A.L.et al [107]), and got FDA approval for its use. Now its use is accepted in various common healing processes worldwide.

Electromagnetic radiation is broadly classified into ionizing and non-ionizing radiation. The study of interaction between non ionizing electromagnetic fields and biological systems is called as Bioelectromagnetics.

Magnetic field therapy included electromagnetic fields consists of six varieties that are developed and used in vast number of countries throughout the world (Markov, M.S. (2004a)et al[108]).

- Static/ permanent magnetic fields created by permanent magnets as well as by passing direct current (DC) through a coil.
- Low frequency sine wave electromagnetic fields use 60 Hz or 50 Hz.
- Pulsed electromagnetic fields are low frequency fields with specific shapes and amplitudes
- Pulsed radiofrequency uses the radiofrequency range 13.76, 27.12. and 40.68 MHz

- Tran cranial magnetic /electric stimulation is a method of treatment of selected areas of the brain with short but intensive magnetic pulses.
- Millimeter waves have very high frequency range of 30-100 GHz.

PEMF has special design and it direct magnetic fields in to the tissues concerned and promote healing. They display broad frequencies in electromagnetic spectrum ranging from 6 Hz up to 500 Hz (C.A. Bassett,et al[109]) .

Use of very low frequency fields are non-ionizing and non heating or minimal heating to tissues (B.Rubik ,et al[110]).The shapes of PEMF wave forms are either asymmetric, biphasic, quasi rectangular or quasitriangular in shape but most are sinusoidal. Specific type produces specific effect; pulsed one has more efficacy than continuous fields (D.H. Trock et al[111]).

PEMF acts on biological systems by two ways:

1. Capacitive or direct coupling in which the opposite electrodes applied directly to skin surface of interest (D.H. Trock et al[111]).

2. Inductive or indirect coupling in which time-varying electric fields are induced at the concerned repair site by the application of a time-varying magnetic field through single or two electrical coils. They are not with skin contact.

The field parameters are determined by the frequency characteristics of the magnetic field applied and also the electrical properties of the target tissue.

2.2.3. Electromagnetic field generation:

"The time –changing magnetic field of the PEMF induces an electric field (Faraday's **law of induction)**, which in turn produces a current in the body's conductive tissue" (D.H. Trock et al[111],G.C. Traina et al[112])

"Faraday law of induction states that a changing magnetic field produces an electric field and if this electric field is in a conducting medium, there will be an electric current flow. This induced voltage is proportional to the time- rate of change of the magnetic field" (Sanker Narayananet al [113]).It means that the potential difference (or voltage, V) between two ends of a wire, placed in a changing magnetic field is $V= -d\Phi/dt$ where the time-rate of change is flux $=d\Phi/dt$.

The electric field induced via a time-varying magnetic field waveform is directly related to the electrical characteristics of the coil employed and the current waveform applied to the coil.

Induced electromotive force (emf) is proportional to the rate of change of current in the coil (dIcoil/dt) which produces the shape of the induced electric field.

Faraday's Law of Induction and Maxwell's equations explains the generation of electromagnetic field (EMF).A static electric field (EF) is generated by a static charged particle. The EF (or E component of an EMF) exists whenever charge is present. Its strength is measured in volts per meter [V/m].

An EF of 1 V/m is represented by a potential difference of 1 V existing between two points that are 1 m apart. The V/m is primarily used to express the intensity of the EMF.

MF (or M component of an EM field) arises from the current flow. Its flux density is measured in tesla (T) in the International System (SI), and in gauss (G) which is the former CGS system of units (1 G = 10-4 T or 1 T = 10 milligauss).

A flux density of 1 G represents 1 Maxwell/cm2. The tesla (or Gauss) is mainly used to denote the flux density (intensity) produced by MFs. Both electric fields (EFs) and magnetic fields (MFs) are generated if a charged particle moves at a constant velocity.

EMFs are generated when a charged particle is accelerated. Most often this acceleration takes place in the form of an oscillation, therefore electric and MFs often oscillate. Change in the electric field (EF) creates a MF, and any change in the MF creates an EF.

This interaction suggests the higher the frequency of oscillation, the more the electric and MFs are mutually coupled. EMF devices include solenoids, Trans cutaneous Electrical Nerve Stimulation (TENS), and Helmholtz coils.

The magnetic energy produced is as little as that of the Earth's magnetic field to more than 10,000 times as powerful.

2.2.4. The mechanism of action of pulsed electromagnetic field on wound healing:

Based on a number of cell and animal studies it was suggested that PEMF acts on Ca2+ calmodulin signaling mechanism leading to nitric oxide (NO) production (Strauch, B. et al[114]) and growth factor cascades involved in tissue healing.

PEMFs modulate the calcium-binding kinetics to calmodulin. Calcium/calmodulin (Ca/CaM) then activates nitric oxide synthase (NOS) in several different isoforms.

When injury occurs, large amounts of nitric oxide are produced by long-lived inducible nitric oxide synthase (iNOS). In this cascade, tissue levels of nitric oxide persist and the prolonged presence of this free radical is proinflammatory, which accounts for the leaky blood vessels associated with pain and swelling.

In contrast, the endothelial and neuronal nitric oxide synthase isoforms (respectively eNOS and nNOS) produce nitric oxide in short bursts that can immediately relax blood and lymph vessels.

These short bursts of nitric oxide also lead to the production of cyclic guanosine monophosphate (cGMP), which in turn drives growth factor production. iNOS is not dependent on CaM, while the constitutive or cNOS (eNOS or nNOS) cascade is dependent on the binding of Ca/CaM.

Therapies that could accelerate Ca/CaM binding, therefore, should impact all phases of tissue repair, from initial pain and swelling to blood vessel growth,

tissue regeneration, and remodeling. As shown in the following diagram, this mechanism has been proposed as a working model for PEMF therapeutics.

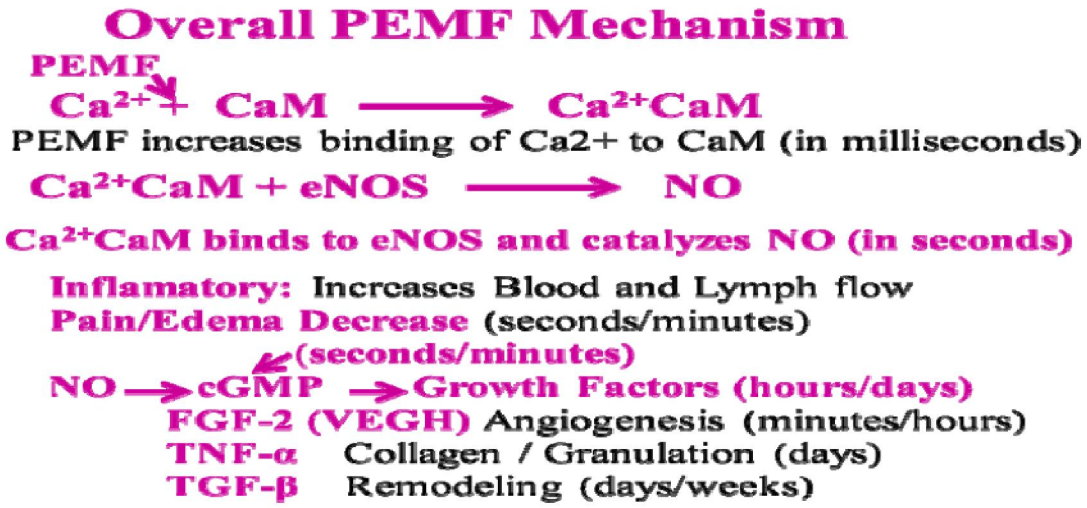


Fig-8.PEMF Mechanism

It is known that NO is a critical molecular signal and mediator for normal wound healing (Boykin, J. V.et al[115],Filippin, L. I.,et al[116]) NO deficiency has been established as an important mechanism responsible for poor healing in diabetic foot ulcer patient.

Co-cultures of human dermal fibroblast and human epidermal keratinocytes exposed to PEMF demonstrated an up- regulation of gene families involved in tissue repair. These include matrix metalloproteinase (MMPs) and tissue inhibitor of metalloproteinase (TIMP's), and cytokines - interleukin (IL)-related genes, interferon (INF)-related genes, and tumor necrosis factor (TNF)-related genes.

2.2.5. Biophysical consideration of EMF therapeutics: The induced current at the wound site leads to several electrochemical interactions at cell surfaces,

- Non specific electrostatic interactions of water dipoles and hydrated (or partially hydrated) ions at the lipid bilayer/aqueous interface of a cell membrane and make all cell membranes a capacitor(Pilla, A. A.,et al[117]).
- Voltage dependent ion/ligand binding (Plonsey R, et[118])→ ion or dipole competes effectively with water dipoles and hydrated ions at specific membrane sites, which modulate a downstream cascade.

The electrochemical pathways involved in the transduction of an exogenous EMF signal operates similar to the initial gating process involved in the production of the action potential via membrane depolarization(Pilla, A. A.et al[119]).

A non thermal EMF has direct affect ion binding and/or transport and influence the cascade of biological processes related to tissue growth and repair (Lee, R. C.,et al [120]).

Most wound studies involve arterial or venous skin ulcers, diabetic ulcers, pressure ulcers, and surgical and burn wounds. Since cells involved in wound repair are electrically charged, some endogenous EMF signals may facilitate cellular migration to the wound area (Markov, M. S., Hazlewood,et al[121]) thereby restoring normal electrostatic and metabolic conditions.

Because the main goal of any therapy is to restore normal function to the organism, electric, magnetic, or electromagnetic modalities appear suitable to

compensate the injury currents. PEMF have also been beneficial in treatment of chronic pain associated with connective tissue like cartilage, tendon, ligaments, and bone injury and joint-associated soft tissue injury .Growth factor activation of the Na-K ATPase enzyme in fibroblasts (Kaplan JG.et al[122]).Ca^{2+} regulation, via CaM, of the cell cycle; Ca^{2+} dependent adenylate cyclase activation in macrophages Whifield JFet al[123]).Ca/CaM regulation of growth factor and other cytokine release (Means AR et al [124]).

EMF could also modulate the distribution of protein and lipid domains in the membrane bilayer, as well as conformational changes in lipid-protein associations by altering the kinetics of binding.

Ion/ligand binding represents a coupling or transduction mechanism for exogenous electromagnetic fields at biological surfaces and junctions, which can be used to quantitatively and predictively configure bioeffective EMF waveforms.

Cellular gap junctions provide pathways for ions and molecular intercellular communication (Sheridan JD, et al[125]).and provide ionic coupling and metabolic cooperation which is crucial for growth control and tissue repair. The role of cooperative organization in the EMF sensitivity of biological systems has been qualitatively considered (Adey WR.et al[126]).Functional modification of gap junctions by modulated microwave fields, as well as EMF signals has been reported (Fletcher WH et al [127]).

In July of 2002, the accumulated background of information led the U.S. Centers for Medicare and Medicaid Services to finally approve coverage for

electrical stimulation as adjunctive therapy for stage III and stage IV pressure ulcers, arterial ulcers, diabetic ulcers, and venous ulcers, providing that improvement had not occurred after 30 days of standard wound treatment"(PJ Rosch,et al[128]).

The interaction between various types of cells (vascular, epidermal and dermal cells), growth factors, cytokines, chemokines, adhesion molecules, nitric oxide, trace elements and proteases usually determine the outcome of normal healing process. The electromagnetic field therapy targets these interactions. Hyperglycemia and disturbances in insulin signaling affect the glucose utilization in keratinocytes, thereby its differentiation and proliferation (Spravchikov N, et al[129]).

Also, reduced nitric oxide synthesis seen in diabetes retards the functioning of keratinocytes and fibroblasts, essential for normal wound healing resulting in impaired wound healing (Shi HP et al[130]).Decreased concentration of platelet activating factor in wound is also associated with impaired healing. Although, many causes for impaired wound healing have been suggested, vascularity of tissues seems to be most important, which is targeted by the PEMF therapy.

PEMF therapy effect on microvascularity:

PEMF accelerates the endothelial cell growth rate (Yen-Patton GP et al [131]). Sinusoidal PEMF was shown to improve the microcirculation, fibroblast proliferation and differentiation (Nikolaev AV, et al[132], Loschinger M et al [133]

Hinsenkamp M, et al[134]).The underlying mechanism of this has been suggested as immediate and temporary rise in cAMP-dependent protein kinase activity (Thumm S, et al[135]).The improvement in blood flow is found mainly small arteries and arterioles in the wound site (Matsuda T et al [136]) .

F. Influence on cytokine production:

Exogenously applied EMF affects cell signaling and cytokine production. In a model studying osteoarthritis, EMF showed statistically significant improvement in stimulating transforming growth factor beta (TGF-β)(Ciombor D,et al[137])

TGF-β upregulate gene expression of cartilage core protein and MMP inhibitors Matrix metalloproteases of (MMP) and IL-1 are down regulated by TGF-β and it was suggested that EMF influences homeostasis of cartilage by stimulating TGF-β. Also some studies show that cytokines IL-1β and TNF-α are decreased and IL-10 is increased by PEMF (Gómez-Ochoa et al [138]).

Some of the effects of PEMF:

1. Increased number of chondrocytes, 75Hz, 2.3 mT (Richards TL et al[139])
2. Influence proliferation of osteoblasts, 15 Hz, 0.1 mT . (Mattei MD et al[140])
3. Osteoclasts shows a decreased production of osteoclasts, 7.5 Hz, 300μs
4. Adenosine receptors are saturated with neutrophils and decreases cytokine cascades75 Hz, 0.2 mT to 3.5 MT (Gomez-Ochoa I et al[138])

5. Mononuclear shows a significant increase in IL-1β and TNFα- proinflammatory cytokines, 50Hz, 2.25mT (Farndale R et al[141])

6. Fibroblasts show cAMP reduction and collagen proliferation leads to increased proliferation of collagen cell, 15Hz, with a pulse of 4.8 ms (Delle Monache S. et al [142])

7. Endothelial cells shows a increased count leading to angiogenesis, 50 Hz, 1 me (Pipitone N et al[143])

2.3.1. "Vascular endothelial growth factor":

Vessels are formed mainly by two consequent mechanisms namely vasculogenesis and angiogenesis.

Vasculogenesis :

Blood vessels are synthesized denovo, from stem cells of mesodermal origin, hemangioblasts that in turn gives rise into primordial hematopoietic cells and endothelial cells called angioblasts.

Angiogenesis, development of new capillaries from preexisting vessels is a highly regulated process, playing a major role in physiological processes like embryological ovulation, menstruation and wound healing (Carmeliet P.et al [144])

Disproportionate angiogenesis is seen in tumors and some other systemic disorders. VEGF receptors are expressed in the cells responsible for angiogenesis, implying the role of VEGF in blood vessel and capillaries formation.

2.3.2. Structure of VEGF:

VEGF is a homodimer, glycoprotein in nature closely resembling platelet derived growth factor (PDGF) in its structure (Keck PJet al[145])

Various forms of VEGF isolated till date includes VEGF-A, VEGF-B, VEGF-C, VEGF-D and placenta growth factor. VEGF is usually referred to as VEGF-A, primarily referred to as Vascular Permeability Factor VPF, owing to its nature of inducing vascular permeability.

Mechanism of action of VEGF:

The actions of VEGF are mediated by tyrosine kinases, namely VEGFR-1 VEGFR-2 and VEGFR-3. The vasculogenic and angiogenic activity of VEGF seen in various physiological processes is mediated by VEGFR-2, primarily found in endothelial cells (Robbins and cotran et al [146]).

VEGF is produced by cells participating in wound healing processes like endothelial cells, fibroblasts, smooth muscle cells. Furthermore, hypoxia, transforming growth factor α and β and Platelet derived growth factor may induce the VEGF production from these cells.

The VEGF has versatile function, acting in a paracrine fashion, affecting the endothelial cells and skin microvasculature. It also plays a role as an endothelial cell mitogen, a chemotactic agent(Ferrara N et al [147]) and wound healing process, mainly angiogenesis, epithelization and collagen deposition (Stojadinovic OKA et al [148])

Mechanism of angiogenesis:

Angiogenesis, otherwise termed neovascularization, involves the recruitment of progenitor cells of endothelium from bone marrow which cause the extensive branching off and extension of pre-existing blood vessels.

Process of angiogenesis from pre- existing vessels:

- Nitric Oxide mediated vasodilation of the parent vessel
- Degradation of parent vessel basement membrane by matrix metalloproteinase (MPS)
- Disruption of cellular contacts between adjacent endothelial cells by plasminogen activator
- Endothelial cells migration towards the angiogenic stimuli
- Proliferation of migrated endothelial cells, followed by the maturation of endothelial cells
- Migration of Pericytes and vascular smooth muscle cells to form the mature blood vessel (Robbins and cotran et al [146]).

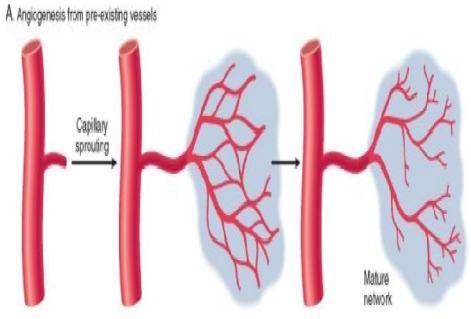


Fig.9. Angiogenesis from pre-existing vessels

2. Process of Angiogenesis from endothelial precursor cells (EPCs):

- Mobilization of endothelial precursor cells from the bone marrow
- Migration of **EPCs** to injury area
- Differentiation, maturation of (EPCs) and formation of mature capillary plexus by forming links with previously existing vessels.

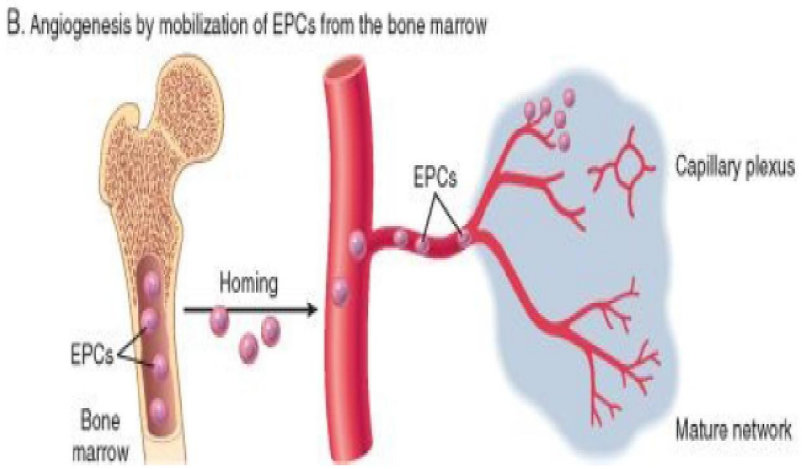


Fig.10. Angiogenesis by mobilization of EPCs from the bone marrow

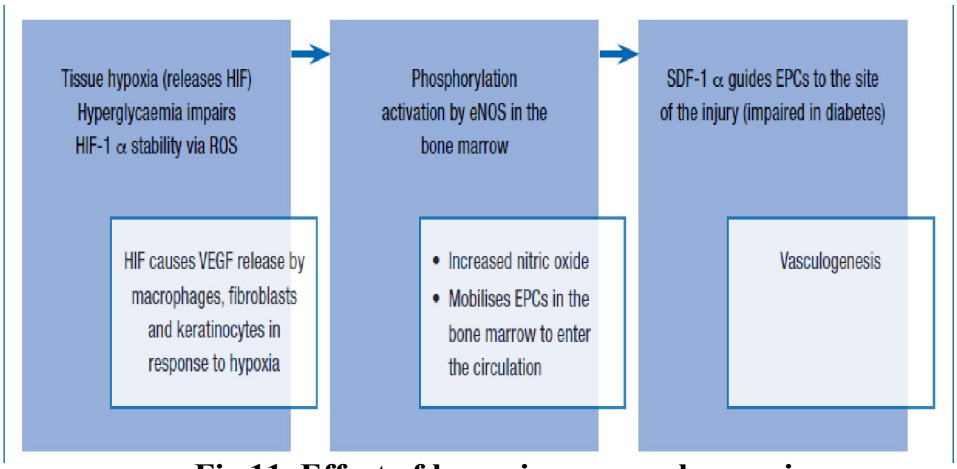


Fig.11. Effect of hypoxia on vasculogenesis

2.3.6. Role of VEGF in angiogenic cascade mechanism:

VEGF has multiple roles in wound healing such as new vessel formation dilation of blood vessel, degradation of basement membrane, Proliferation and migration of endothelial cells(Folkman, J et al [149])Capillary tubes are formed that anastomose as parallel sprouts , loops and new basement membrane are formed consequently.

1. Vasodilation:

VEGF leads to vascular permeability by stimulating nitric oxide synthase (NOS) and increase NO synthesis. Which in turn increase vessel permeability and dilation.These changes in the vasculature increases growth factors sensitivity as well as VEGF expression in a positive feedback fashion(Folkman, J et al [149], Murohara T et al[150])

2. Basement membrane degradation:

VEGF increases MMP-1, MMP- 2 and other collagenases by acting directly on endothelial cells. The resulting proteolytic enzymes destroys extracellular matrix and basement membrane favors endothelial cell migration.

3. Migration of endothelial cells:

VEGF leads to chemotaxis of cells and blood vessel dilatation

4. Proliferation of endothelial cells:

VEGF is act as a "mitogen" to endothelial cells selectively. Nitric oxide and cyclic GMP are involved in this signalling (Morbidelli L,et al[151])

Endothelial cell proliferation and its invasion into collagen matrix is aided by VEGF (Goto Fet al [152]). "VEGF lengthens the life span of endothelial cells and prevents apoptosis. There are some studies showing temporary anti-apoptotic protein expression in human endothelial cells" (Gerber HP et al [153])

5. Wound Healing and VEGF:

Granulation tissue formation is vital for normal wound healing and it is "the hallmark of an established healing response". Angiogenesis where new vessels are formed depend on. "Inhibition of angiogenesis impairs wound healing" (Brem H,et al[154])

6. VEGF and Diabetic foot ulcers:

Though diabetic milieu favors VEGF expression, animal studies with full thickness excisional wounds showed early increase in VEGF levels which then drop to untraceable levels (Frank S et al [155])

Diabetic wounds show increased levels of several growth factors like VEGF on exposure to PEMF. VEGF promote chemo taxis, angiogenesis, and enhance healing. Nitric oxide synthesis is facilitated by PEMF application and it mediates VEGF activity and deposition of collagen in diabetic wounds. Also it

improves function of endothelial cells, increase tissue oxygenation and nerve conduction. PEMF can be used an ideal adjunct therapy for chronic non healing diabetic foot ulcers.

Some of clinical application of PEMF:

A study by Bassett et al[156] in Beagle dogs showed enhanced bone repair.

Wilson & Jagadeesh et al[157] in rats with diapulse; 65μsec bursts,80-600pulses/sec showed increase of nerve regeneration.

Bassett et al[158] by quasi-rectangular, assymetrical, 300μ pulse width of 75 Hz 12-16 hrs daily for 3-6 months showed osteogenesis.

Heckman et al[159] ElectroBiology study with quasi-rectangular, assymetrical pulses showed 64.4% of healed ununited fractures.

Binder et al[160]with 73 + 2 Hz; 2.7 mT (peak) 5-9 hrs daily for 4 weeks reduced pain and improved active range of movements.

Raji[161] in Rats with diapulse 400 pulses/sec 15 min daily for 3.5 days, 1, 2, 3, 4, or 8 week accelerated the rate of recovery of injured nerve, enhanced regeneration of damaged nerves

Sisken et al[162] in a rat study showed sciatic nerve generation.

Ieran et al[23] showed increase success rate of treating venous skin ulcers; reduced recurrence rate

Stiller et al[163] with PELUT,ΔB showed decreased wound depth and pain intensity

Kaszuba-Zwoińska Jolanta et al[164] in human bladder microvascular endothelial cell line (HMEVEC-Bd) and MonoMac6 (MM6) cells with 7Hz, 30mT for three times with 24h intervals showed decrease of measured cell death parameters diminished production of some inflammatory agents.

AIM AND

OBJECTIVES

Aim and Objectives

The aim and objectives of this thesis includes

Aim: Evaluate of effect of "**Pulsed electromagnetic field therapy"** in promoting healing in chronic non healing diabetic foot ulcers

Objectives:

1. To administer Pulsed Electro Magnetic Field therapy to the Diabetic Chronic foot ulcer patients
2. To assess and compare healing parameters after Pulsed Electro Magnetic Field therapy
    i. Wound size and dimensions after the treatment
    ii. Healthy granulation tissue formation after treatment
    iii. Level of exudation after treatment
    iv. Serum vascular endothelial growth factor after the treatment

MATERIALS AND METHODS

Methods and materials

This study was conducted during the period of Sep2014- Aug 2015 at the Institute of Diabetology and Institute of surgery Rajiv Gandhi Government General Hospital Chennai after Institutional Ethics Committee (IEC) clearance is obtained from Madras Medical College.

Selection of subjects:

Thirty patients both men and women in the age group of 40-60 years were selected from the department of Diabetology and surgery, Rajiv Gandhi Government General Hospital. Diabetic patients, both Type 1 and 2 with foot ulcers of Wagner's grading 1 and 2 of duration more than 4-6 weeks duration participated in the study. Informed consent was obtained from all the patients and they were explained about the daily treatment protocol and follow up schedule. They were instructed to continue anti diabetic treatment throughout the study period.

Inclusion criteria:

- Men and women of Age group 40-60 yrs
- Diabetic patients
- Grade1 &2 [Wagner's] foot ulcers
- Duration of foot ulcers more than 4-6 weeks.

- Good glycemic control
- Neuropathic ulcers

Exclusion criteria

- Uncontrolled DM
- Wagner's grade 3,4,5 ulcers
- Severely Infected wounds and Gangrene
- Neuroischemic ulcers, traumatic ulcers
- Peripheral vascular disease
- Coronary artery disease
- Varicose veins
- Deep Venous Thrombosis
- Malignancy
- Pacemakers

Study Design:

Prospective observational Case Control Study

Study centre:

Department of Diabetology & Surgery

Rajiv Gandhi Government General hospital, Chennai-3

Pulsed magnetic field therapy:

The equipment consists of a controlled magnetic field system-portable type along with its accessories-a signal generator [Make systronics, model 1012, and sr. No 4588] and a monitoring meter assembly [oxford 50-0-50mA range]

"The coil assemblies, designed and fabricated at **Madras Institute of Magneto biology** are carefully calibrated using high precision magnetometers and current measuring devices in the Magnetic Standardization Lab of the institute. The high precision measurement involves calibration of the coils for arriving at the coil constant expressed in nano- Tesla [nT] per milli –ampere [mA] of current".

The signal generator delivering the current to the coil assembly is capable of operating at 110/120 V, 50Hz at the primary end and at the secondary end deliver currents [in mA in the range of 10-100] at desired waveforms [typically sine ,square, and ramp] in varying frequencies [between 0.1 Hz to 1000Hz at voltages between 0.2 to 20 volts] .The meter to measure the current delivered to the coil from the signal generator is a class 2 type [pre calibrated] milli-ammeter.

Pulsed electromagnetic magnetic fields are generated within special controlled magnetic field enclosures [coil system] by sending measured amount of electric current whose amplitude, frequency and wave form can be controlled using signal generators.

These design guidance equations have been made use of by **Fanselau and Braunbeck** for developing a system of four coils which have the following geometry in their mounting.

The assembly consists of two outer [smaller] coils of radius a_1 spaced $2d_1$ apart and within these are mounted two inner [larger] coils of radius a_2 with spacing between them = $2d_2$, all these bearing specific relation with respect to a_1 , the coils being of course coaxial and co-planar and carrying the same number of turns with a single turn of wire in each of these four coil forms all connected in "series –aiding " the system will give a field,

$$H = \frac{[100 \times 1(\text{number of turns in each coil being}=1)}{0.80852 \times a_1 \text{ (where } a_1 \text{ is expressed in cms)}}$$

for 1millampere of current flowing through them.

With this assembly, the generated field can be expected to be uniform to as good as about 1 part in 5000 or so inside the entire disc shaped volume between the two inner [larger] coils of radius, a_2.

The four coils form are wound with the two separate sets of windings : a "Steady Field" winding of 25 turns in each coil and a "pulsation" winding of 6 turns in each coil , the windings being "series –aiding." The steady field winding gives a coil constant of 40,000nT per ampere of current and the pulsation winding, 9.6nT per milliamp.

The entire coil assembly is mounted on bearings ,permitting the coil axis to swing through 360° in the local magnetic meridian .With this system, by passing appropriate DC through the steady field winding and with appropriate angle of dip for the coil axis , one can generate inside the coil assembly, magnetic field of any spot on earth [in intensity and direction].

The system can also be used for creating a magnetic vaccum inside , by dipping the axis at the local magnetic inclination or Dip (+12° for Chennai) and sending a current of the right value and direction to cancel the local GMF of about 37,000nT. The pulsation winding can be independently energized by a function generator to provide a pulsed magnetic field of any desired frequency, intensity and waveform inside the enclosure.

The area /body part to be exposed to the pulsed magnetic fields generated inside the coil system is to be placed within the region of most uniform magnetic flux density for the prescribed duration of exposure.

Pic-1 (Portable PEMF unit with signal generator)

Pic-2(Patient with PEMF Unit)

PEMF Safety:

- The device provides an ultra low intensity (1500nTesla) which is about $1/40^{th}$ of normal earth's magnetic field at extremely low frequencies (ranging between 1 to 10 Hz) for therapy.
- This ensures extreme safety to the patients
- No radiation is involved
- It is totally non-invasive
- Neither medications (internally or externally administered) nor injections
- No side effects

Proposed treatment protocol for a duration of 45 min/day for 30 days

- Low current strength of 30 m A
- Frequency of 10 Hz
- Wave form of Sine wave
- Intensity of 1500nT
- One day break after every 6 days

The patients were screened for:

1. **Sensory loss:**

   Is accessed by "**Modified neuropathy disability score (NDS)".** It is a simple, clinical assessment tool which gives quick results. It aims at

combination of a number of clinical tests and provides an assessment of the neuropathic ulceration risk. Each foot is tested separately and scored independently and the results were added together.

A score of 10 is said to be maximum which indicates complete loss of sensation

Score of 6 and more indicate moderate or severe neuropathy

1.Vibration Perception Threshold Tuning fork (128Hz) is applied over the apex of the big toe	**Score 0**	**Score 1**	
	Normal	**Abnormal**	
2.Perception of Temperature Beaker of warm or cold water applied over the dorsum of the foot	**Score 0**	**Score 1**	
	Normal	**Abnormal**	
3.Pin prick test Sharp pin applied proximal to the big toe nail	**Score 0**	**Score 1**	
	Normal	**Abnormal**	
4.Achilles tendon reflex knee hammer tapped on the Achilles tendon	**Score 0**	**Score 1**	**Score 2**
	Present	**Present with reinforcement**	**Absent**

“Biothesiometer or Neurothesiometer:

This device is simple and hand held that assess vibration perception threshold (VPD) semi quantitatively. The patient is comfortably lies in supine position and the stylus of the instrument placed on the plantar aspect. Six points over plantar surface were checked and the amplitude is increased gradually till the patient detect the vibration and the resulting number is called as the VPT.

VPT more than 15 are regarded as abnormal and higher values of VPT “strongly predictive of sensory loss”.

II. Assessment of Biomechanics:

Presence of Callus graded as **Yes**

Absence of Callus graded as **No**

III. Vascular assessment:

1. **Palpation of posterior tibial artery** below and behind the medial malleolus bounding pulse

2. **Palpation of dorsalis pedis artery** lateral to the extensor hallucis longus tendon on the dorsum of foot bounding

Pic-3(Biothesiometer)

Pic-4 (Assessment of pain)

Pic-5 (Assessment of vibration sense with128Hz tuning fork)

Pic-6(Ankle jerk)

Pulses if present graded as yes, not present as No

3. **Ankle Brachial pressure index**:

A hand held Sphygmomanometer is used

ABI measured by dividing the ankle systolic pressure by the brachial systolic pressure.

ABI >0.9 to <1.3 is normal

IV. Ulcer healing parameters:

1. Wound Length and Width - longest and widest aspect of the wound surface measured with ruler in cms

2. Wound Surface area in cm^2 or **Size**

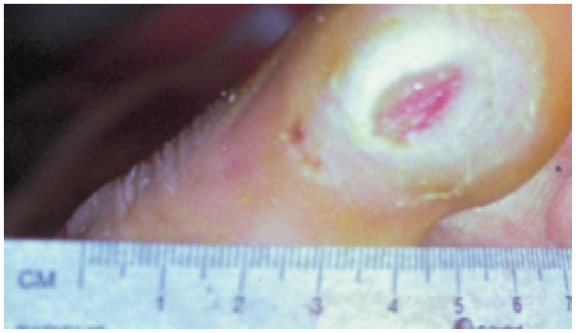
3. Percentage reduction in surface area as calculated as

$$\frac{\text{Initial surface area – final surface area X100}}{\text{Initial surface area}}$$

4. **Depth:** Measured by thin sterile metal probe placed perpendicular into the lowest section of the wound marked with marker pen read with a ruler in cms

Pic7 (Vascular Assessment)

Pic-8 Butter or tracer paper spread on the wound to measure exudates amount



Pic-9(Ulcer measurement with ruler)

5. Tissue type: "This refers to the type of tissue that is present in the wound (ulcer) bed and scored as: (4) – Necrotic Tissue (Eschar): black, brown, or tan tissue that adheres firmly to the wound bed or ulcer edges and may be either firmer or softer than surrounding skin. (3) – Slough: yellow or white tissue that adheres to the ulcer bed in strings or thick clumps, or is mucinous.(2) – Granulation Tissue: pink or beefy red tissue with a shiny, moist, granular appearance.(1) – Epithelial Tissue: New pink or shiny tissue (skin) that grows in from the edges or as islands on the ulcer surface.(0) – Closed/Resurfaced: the wound is completely covered with epithelium (new skin)"

Tissue Type Scores	
Tissue Type	**Score**
Presence of any necrotic tissue	4
Any amount of slough present Absence of necrotic tissue	3
Clean wound Granulation tissue present	2
Wound is superficial Re-epithelializing tissue	1
Wound closed	0

6. Level of exudation is calculated by placing a butter (or) tracer paper spread on to the wound area and amount of soaking is seen and scored as

Exudation Scores	
None	0
Light	1
Moderate	2
Heavy	3

Grading of tissue type and exudates amount is followed from

Pressure Ulcers: Scale for Healing (PUSH)

V. Probe to bone test: Done with a sterile metallic probe to rule out osteomyelitis.

VI. Serum VEGF levels assessed before and after PEMF therapy

Blood samples are collected, serum separated by centrifuging and stored at desired temperature.

Assessment of serum VEGF:

VEGF ELISA (Enzyme-Linked Immunosorbent Assay) kit was used to assess serum VEGF levels. This procedure is an in vitro enzyme –linked

immunosorbent assay and it measures humanVEGF levels in the serum quantitatively.

Principle:

This assay is based on the principle of using an antibody that is specific for human VEGF which is coated on a 96-well Elisa plate. The standards and samples were pipetted into the wells and if VEGF is present in the sample it will get bound to the wells by the immobilized antibody. The wells were washed and then added with biotinylated anti-human VEGF antibody.

Unbound biotinylated antibody also washed then HRP-conjugated streptavidin was pipetted into to the wells. The wells were again washed, a TMB substrate solution then added to the wells. There will be development of a colour in proportion to the amount of bound. Then stop solution is added which changes blue colour to yellow, and the intensity of the colour developed is measured at a wave length of 450 nm.

II. The reagents provided with Kit

1. "VEGF-A Microplate (Item A): 96 wells (12 strips x 8 wells) coated with anti-Human VEGF-A.

2. Wash Buffer Concentrate (20X) (Item B): 25 ml of 20X concentrated solution.

3. Standard Protein (Item C): 2 vials of Human VEGF-A. 1 vial is enough to run each standard in duplicate.

4. Detection Antibody VEGF-A (Item F): 2 vials of biotinylated anti-Human VEGFA (each vial is enough to assay half microplate).

5. HRP-Streptavidin Concentrate (Item G): 200 µl 300X concentrated HRPconjugated streptavidin.

6. TMB One-Step Substrate Reagent (Item H): 12 ml of 3,3,5,5' tetramethylbenzidine (TMB) in buffer solution.

7. Stop Solution (Item I): 8 ml of 0.2 M sulfuric acid.

8. Assay Diluent A (Item D): 30 ml of diluent buffer, 0.09% sodium azide as preservative.

9. Assay Diluent B (Item E): 15 ml of 5X concentrated buffer".

The Materials required are:

1. A microplate reader which is capable of measuring the absorbance at a wavelength of 450 nm.

2. Precision pipettes that deliver two microlitre to one millilitre volumes.

3. 1-25 ml adjustable pipettes for preparation of the reagent

4. Graduated cylinders. 100 ml and 1 litre

5. Absorbent paper.

6. Distilled water or deionized water

7. Computer and software for ELISA data analysis or Log-log graph paper

8. Test tubes for preparing sample or standard dilutions

Assay procedure:

1. Reagents, samples and standards were prepared as instructed.

2. 100 µl of standard or sample are added to each to well then incubated for 21/2 hour at room temperature or at 4° C for overnight

2.100 µl of prepared biotin antibody is added to each well then incubated for 1hour at the room temperature.

3.100 µl of prepared Streptavidin solution added and incubate for 45 minutes at the room temperature.

4.100 µl of TMB One -Step Substrate Reagent is added to each well and incubated for 30 minutes at the room temperature.

5. 50µl of Stop Solution is added to each well then read immediately at the wavelength of 450 nm

Results Calculations:

The mean absorbance is calculated for each set of samples duplicate standards and controls. The average zero standard optical density is subtracted. The standard curve then plotted on a log -log graph paper or using Sigma plot software. Standard concentrations were drawn on the x-axis and absorbances were drawn on the y-axis.. All the results were calculated and mean and standard deviation was estimated.

Pic-10 (VEGF KIT AND ELISA READER)

Pic-11(Ulcer Before and after treatment)

The minimum detectable dose of Human VEGF-A was determined to be 10pg/ml

Statistical Analysis:

The data collected were subjected to Statistical analysis using the software SPSS version 21. All the results are calculated and mean and standard deviation is estimated. **Paired t' test** was carried out to compare the means of variables between pre treatment and post treatment

RESULTS

RESULTS

Table I	
Demographic data and base line data (n)=30	
Mean Age (years)	55.1±5.01
Age range (years)	43-60
Number of men	23
Number of women	7
Mean duration of Diabetes(yrs)	7.8±1.47
Mean ulcer duration(months)	4.9±1.2
FBS	118.2±7.31
PPBS	147.7±13.9
Systolic BP	128.3±9.499
Diastolic BP	84.867 ±4.8904

With Callus foot	6	
Wagner's classification	Grade 1	Grade 2
	16	14
Ulcer location	Plantar	14
	Heel	11
	Toes	2
	Leg	3

The patients demographic data given in the table 1 shows that the study group included thirty patients (M: F, 23:7) with mean age of 55.06±5.01 yrs. The mean duration of Diabetes is 7.8 ±1.47 yrs and the mean duration of ulcer is 4.9±1.2 months.

Sixteen were grouped under Wagner's 1 and fourteen are grouped under Wagner'2 grading. Among them fourteen had plantar ulcers, eleven had heel ulcers, two had toes ulcers and three had leg ulcers showing the more prevalence of plantar ulcers in this study group. Six patients had callus foot.

None of the patients were not complain of adverse effects and their compliance was good throughout course of the treatment.

The patients showed overall 66% ±15% reduction in the wound surface area. Less than 50 % reduction is reported in 2 of the patients. About 50-59% reduction is noted in 6 patients, 11 patients showed 60-69% reduction, 7 patients showed 70-79% reduction, 2 patients more than 90% reduction and 2 patients showed 80-89% reduction in the wound area.

None reported any worsening of the symptoms.

TABLE-2

Comparison of healing Before and after treatment

VARIABLE	Group	N	Mean	SD	P-VALUE
LENGTH	pre	30	2.5	1.2	0.0004**
	post	30	1.5	0.8	

** P – Value < 0.000 Very Highly Significant

The wound length shows very high statistically significant reduction at the end of the therapy from the base line value (** P – Value < 0.000)

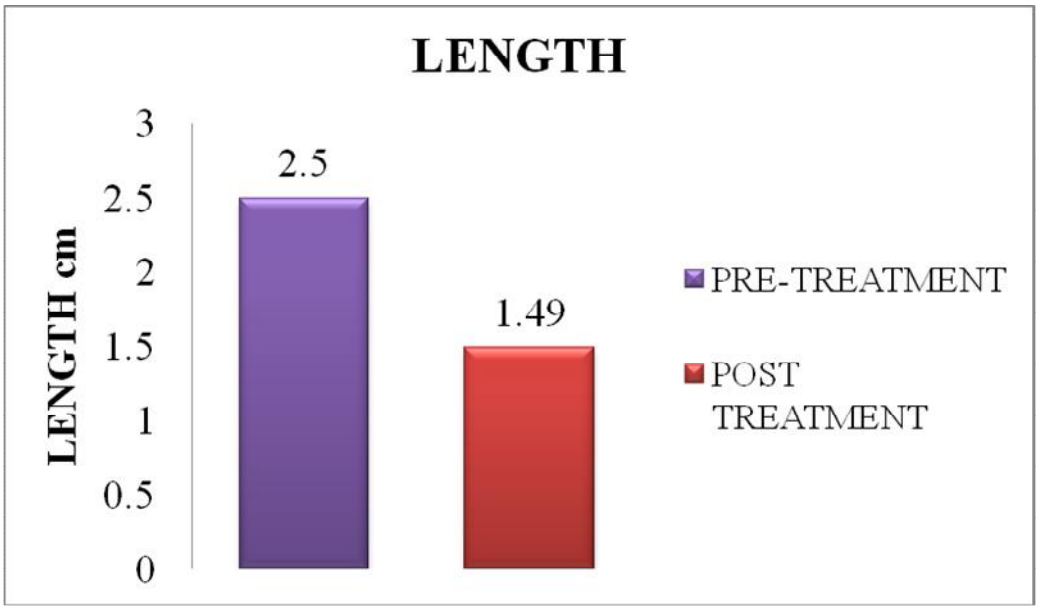
TABLE-3					
Comparison of healing Before and after treatment					
VARIABLE	Group	N	Mean	SD	P-VALUE
WIDTH	pre	30	1.47	.60	0.0001**
	post	30	.8	.40	
** P – Value < 0.0001 Very Highly Significant					

The wound width shows very high statistically significant reduction at the end of the therapy from the base line value (** P – Value < 0.0001)

TABLE-4					
Comparison of healing Before and after treatment					
VARIABLE	Group	N	Mean	SD	P-VALUE
SURFACE AREA	pre	30	4.2	2.8	0.0001**
	post	30	1.54	1.0	
** P – Value < 0.0001 Very Highly Significant					

The wound surface area shows very high statistically significant reduction at the end of the therapy from the base line value (** P – Value < 0.0001)

Graph.1. Comparison of means of ulcer length before and after PEMF



Graph.2. Comparison of means of ulcer width before and after PEMF

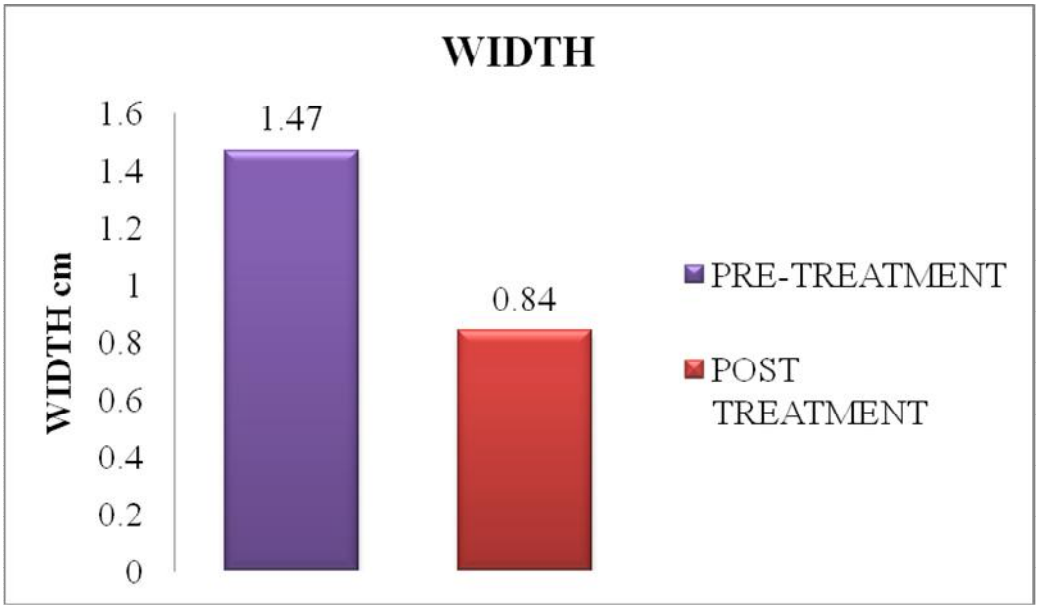


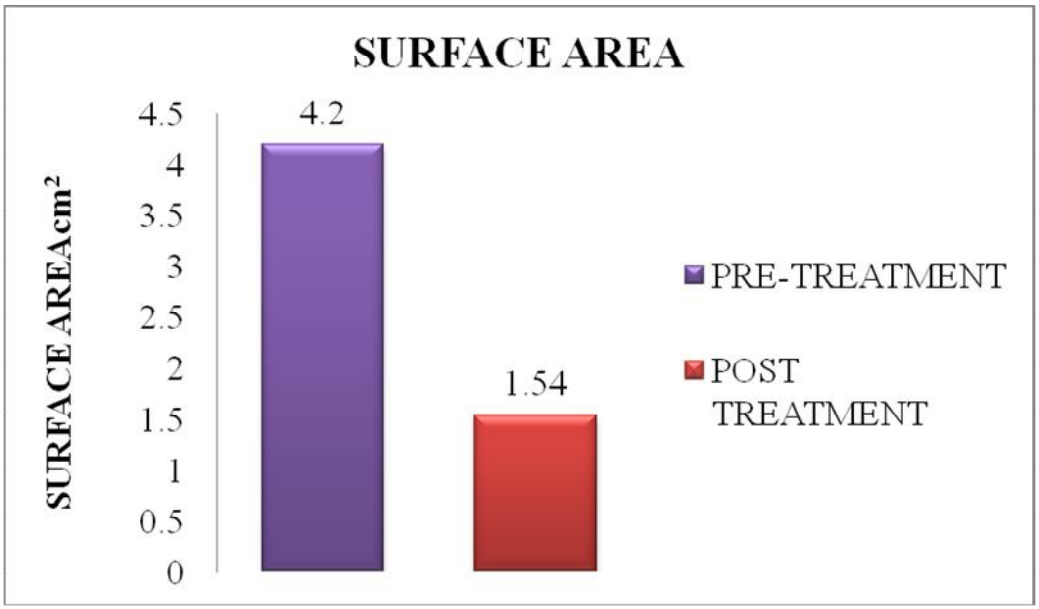
TABLE-5					
Comparison of healing Before and after treatment					
VARIABLE	Group	N	Mean	SD	P-VALUE
DEPTH	pre	30	0.66	.19	0.0001**
	post	30	0.33	.2	
** P – Value < 0.0001 Very Highly Significant					

The wound depth shows very high statistically significant reduction at the end of the therapy from the base line value (** P – Value < 0.0001).

TABLE-6					
Comparison of healing Before and after treatment					
VARIABLE	Group	N	Mean	SD	P-VALUE
TISSUE TYPE	pre	30	3	0	0.0001**
	post	30	1.76	0.61	
** P – Value < 0.0001 Very Highly Significant					

There is very high statistically significant change in the tissue type at the end of the therapy from the base line value (** P – Value < 0.0001)

Graph.3. Comparison of means of ulcer surface area before and after PEMF



Graph.4. Comparison of means of ulcer depth before and after PEMF

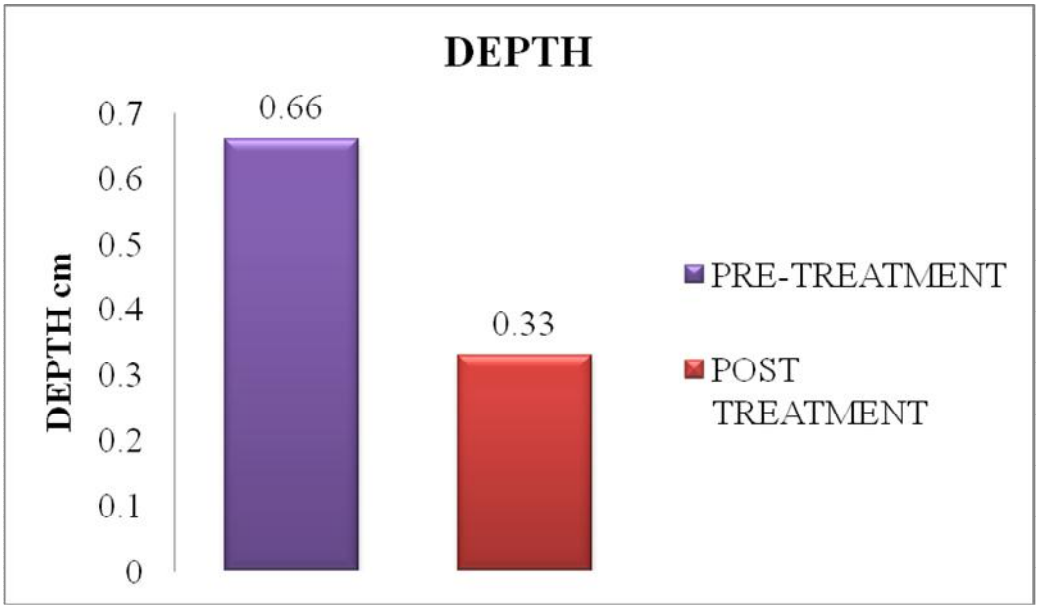


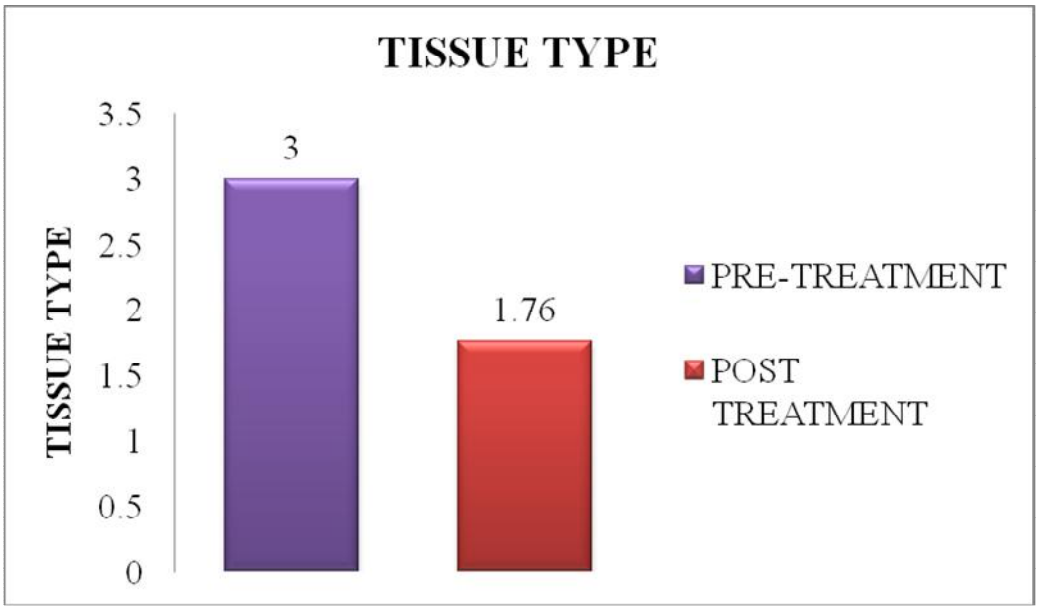
TABLE-7					
Comparison of healing Before and after treatment					
VARIABLE	Group	N	Mean	SD	P-VALUE
EXUDATE AMOUNT	pre	30	1.4	0.48	0.0001**
	post	30	0.16	0.37	
** P – Value < 0.001 Very Highly Significant					

There is very high statistically significant decrease in exudation at the end of therapy from the base line (** P – Value < 0.0001)

TABLE-8					
Comparison of serum VEGF βg/ml levels Before and after treatment					
VARIABLE	Group	N	Mean	SD	P-VALUE
VEGF	pre	30	33	12.4	0.0001**
	post	30	639.1	238	
** P – Value < 0.0001 Very Highly Significant					

There is very high statistically significant increase in serum VEGF levels at the end of therapy from baseline (** P – Value < 0.0001)

Graph.5. Comparison of means of ulcer tissue type before and after PEMF



Graph.6. Comparison of means of ulcer exudate before and after PEMF

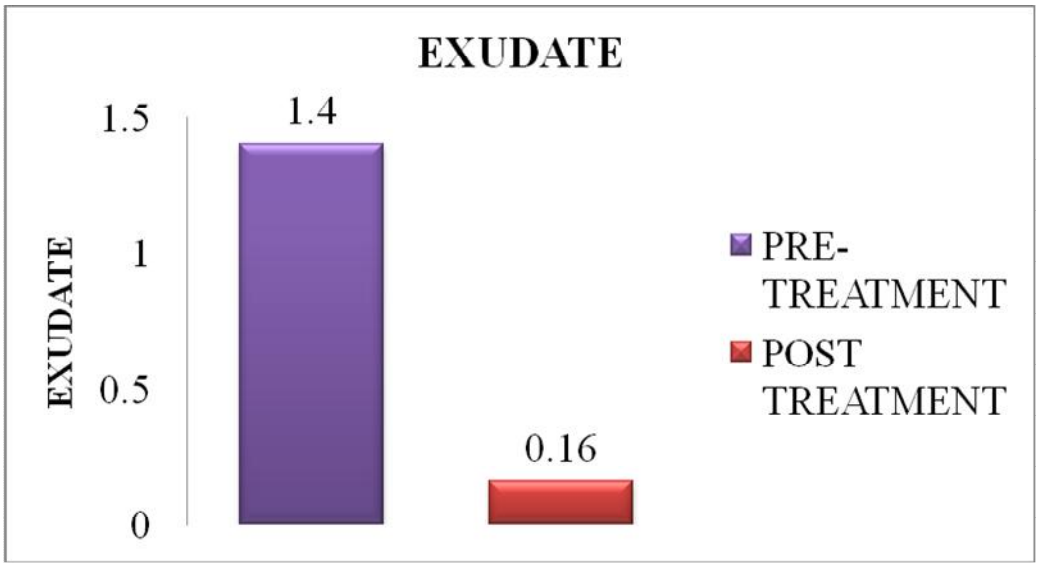


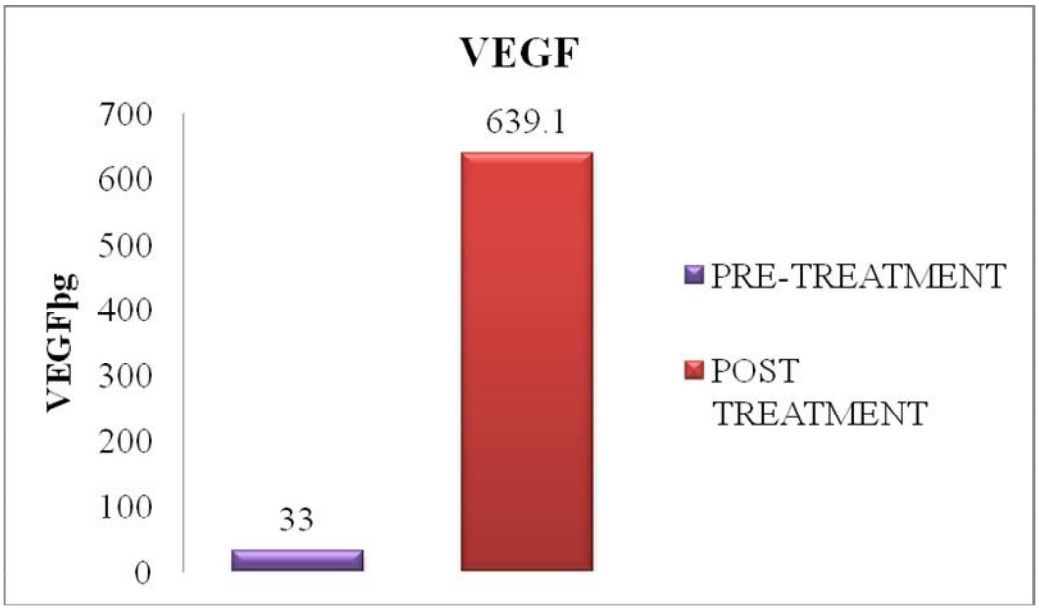
TABLE-9					
Comparison of NDS Before and after treatment					
VARIABLE	Group	N	Mean	SD	P-VALUE
NDS	pre	30	7.3	0.915	0.0001**
	post	30	2.2	0.664	
** P – Value < 0.0001 Very Highly Significant					

There is very high statistically significant reduction in NDS values at the end of therapy from baseline (** P – Value < 0.0001)

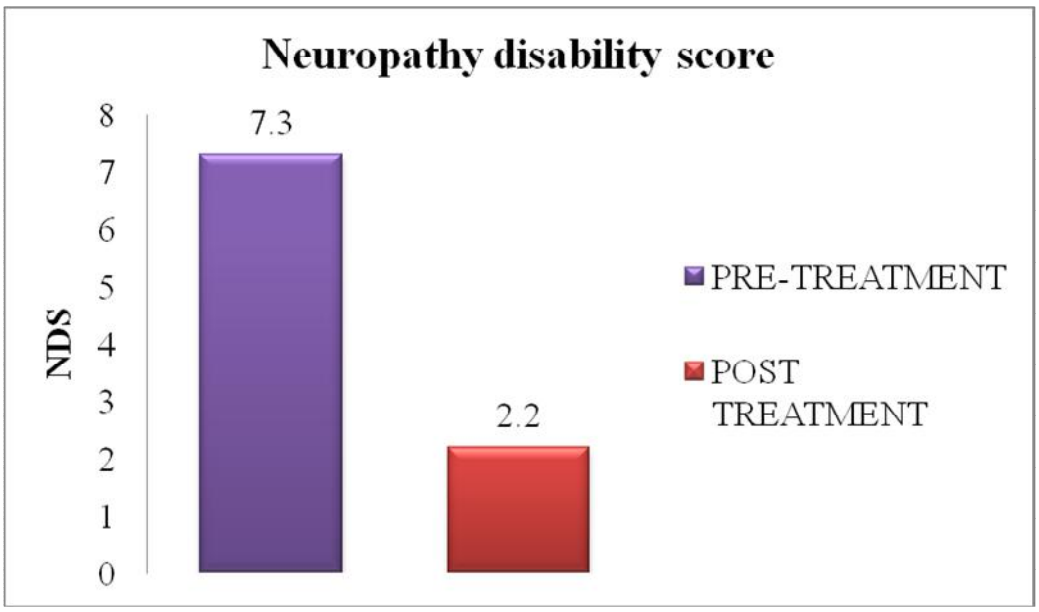
TABLE-10					
Comparison of Biothesiometer Before and after treatment					
VARIABLE	Group	N	Mean	SD	P-VALUE
BIOTHESIOMETER	pre	30	25	3.1	0.0001**
	post	30	12.16	2.53	
** P – Value < 0.0001 Very Highly Significant					

There is very high statistically significant reduction in Biothesiometer values at the end of therapy from baseline (** P – Value < 0.0001)

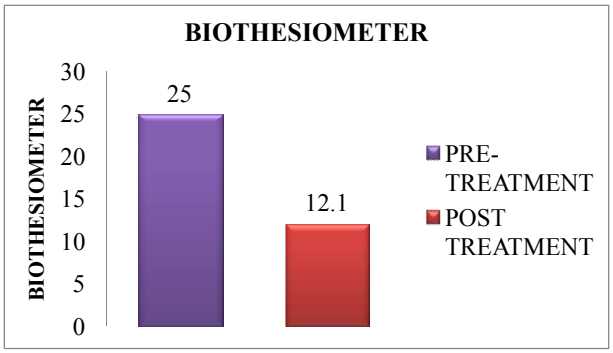
Graph.7. Comparison of means of ulcer VEGF levels before and after PEMF



Graph.8. Comparison of means of ulcer NDS before and after PEMF



Graph.9. Comparison of means of vibration perception threshold before and after PEMF



DISCUSSION

DISCUSSION

This study was conducted to evaluate the effect of Pulsed Electromagnetic Field Therapy in chronic non healing diabetic foot ulcers.

Healing was assessed by

- Reduction in wound size and dimensions
- Formation of healthy Granulation tissue
- Reduction in level of exudation
- Increase in serum VEGF levels

Though debridement and off loading are the conventional methods to treat chronic diabetic foot ulcers their efficacy remains uncertain. This raised the need for intervention with PEMF.

Reduction in wound size and Dimensions:

Treatment with low frequency low intensity Pulsed Electro Magnetic Fields for 45 minutes/day for 30 days showed significant reduction in wound length, width, surface area which is consistent with the study by stiller et al[163].

Kenkre JE et al[165] conducted a randomized trial of case control study in primary care venous leg ulcers. Exposure to electromagnetic fields for 30 days has resulted in reduction in mean ulcer area and some ulcers completely healed.

A study by Ieran et[42] al pulsed electromagnetic field therapy for a duration of 90 days reported significant healing in venous ulcers.

Formation of healthy granulation tissue:

There was a change in tissue type from slough to granulation tissue in almost all the patients showing improvement in healing which is consistent with the study by stiller et al[163].

Ian M Rawe et al[166] observed a steady decrease in wound size as well as decrease in visible peri wound edema.

In both the studies of Stiller et al[163] and Ian M Rawe et al[166] all the patients were well tolerated therapy without any adverse effects.

This study showed that PEMF therapy is safe and effective in healing chronic diabetic foot ulcers.

Canaday and Lee et al[167] have proved that PEMF accelerated wound healing in chronic ulcers.

PEMF therapy increase cellular metabolism and hence increase mitosis of various cells such as fibroblasts and endothelial cells by applying certain frequencies and intensity. This could be the reason for the improved healing of soft tissue in this study (Brette wade et al[168])

There are many in vitro studies showing diverse cellular responses to PEMF which are relevant to wound healing.

Fibroblasts and epithelial cell culture, on exposure to electric fields migrated perpendicular to the applied field. This could be the reason for healing in this study which involves the migration of fibroblasts and epithelial cells to the wound site.

For normal immune system an acute inflammation is necessary. But chronic inflammation seen in diabetes can be detrimental. PEMF able to treat this condition as a non-invasive, low-cost, easy-to-use complement or alternative to currently prescribed treatments (Christina L. Ross et al [169])

Improvement in Sensation:

A significant reduction in **Neuropathy Disability Score** and Vibration Perception Threshold observed is consistent with the study by Wintrobe et al[170] and E.Bosi et al[171].

In a study by Wintrobe et al[170] with pulsed electromagnetic field therapy there was a reduction in diabetic neuropathic pain and stimulated neuronal repair, fourty five percent reduction in DPN symptoms and twenty-nine percent of them had increase in distal leg ENFD (epidermal nerve fiber density) in PEMF group while nil results in sham group.

E. Bosi et al[171] showed a significant reduction in day time and night –time VAS pain score, improved perception of vibration sense and nerve conduction in motor neurons.

The improvement in sensory perception as evidenced by reduction in NDS scores and Vibration Perception Threshold could be due to release of vaso active factors. These resulted in an increase endo-neural blood flow.

Vascular endothelial Growth Factor:

Usually diabetes is associated with a decrease in growth factors, especially **VEGF** levels (Veves A et al[172]).

This study showed a elevation in serum VEGF levels which is consistent with an animal study by Meade et al showing the effect of Pulsed Electro Magnetic Field (PEMF) upon fracture healing in diabetic rats. The rats were treated with PEMF had significant elevated levels of PDGF, IGF-1 and VEGF levels in the fracture callus which showed the importance of growth factors in early healing.

Angiogenic growth factors in bone marrow are up-regulated by Pulsed Electro Magnetic Fields stimulation by in an animal study showed no observed difference in the levels of VEGF with PEMF treated mice but PEMF led to an increase in FGF-2 and Ang-2 but decreased VEGF in mice femoral medulla.

In vivo study by OrenM.et al, matrigel plug assay exposed to electromagnetic fields showed in growth of vessels after 2weeks of exposure to PEMF.

Low dose pulsed magnetic fields to human umbilical vein endothelial cells has resulted in endothelial cell proliferation and endothelial tubule formation.

The ability of PEMF to increase cellular proliferation was unique to endothelial cells. This suggests that endothelial cells are a primary target of PEMF stimulation, releasing protein in a paracrine fashion to induce changes in neighboring cells of different lineages and up-regulating angiogenesis. This could be the reason for elevation of VEGF in this study.

In an another study by Chang-Ning Hao et al[168] found that induced myocardial infarction on exposure PEMF increased density of the capillaries, elevated vascular endothelial growth factor.

In vitro studies with umbilical vein endothelial cells showed that PEMF lead to tubulization and increased Nitric oxide and VEGF secretion and also increased endothelial precursor cells.

Smith et al used a PEMF with positive and negative 18.8 T/s and examined acute changes in arteriole diameter for 2 minutes exposure which showed nine percent increase in the diameter which is increased further on continuation for another 60 minutes.

In a study by Cecilia Y Webb et al[169] showed that treatment with magnetic fields of low intensity and low frequency resulted in significant increase in blood flow and oxygen tension.

CONCLUSION

CONCLUSION

This study concluded that PEMF used effectively as a safe adjunct treatment modality in chronic diabetic foot ulcers.

From various cellular, animal experiments and from human studies it is evident that PEMF therapy linked positively with wound healing processes and outcomes. It is also suggested that its use has relieved wound pain and improved neuropathy in diabetic patients.

Further studies showed elevation in growth factors such as VEGF.

None of the cases reported with any adverse effects.

Easy application, lack of adverse effects, proven clinical and case studies suggest this intervention used as an adjunct therapy for treating diabetic wound foot ulcers.

Further studies with randomized control trials are needed to evaluate PEMF therapy for diabetic foot ulcers.

SUMMARY

SUMMARY

To summarize, diabetes is a common metabolic endocrine disorder emerging as an epidemic throughout the world and is characterized by various dreadful complications.

One such dreaded complication diabetic poly neuropathy leads to foot changes, sensory deficit and micro vascular changes resulting in diabetic foot ulcer.

Once developed it is very difficult to treat them due to repeated trauma, infection and ischemia. The deformities that develop in the foot interfere with normal gait, loss of soft tissue support in plantar aspect leads to pressure points. All these changes finally result in chronic ulcer that fails to heal.

The chronic nature of diabetic foot ulcer is due to arrest of inflammation, loss of balance between extracellular matrix degradation and deposition, defective angiogenesis and failure of growth factors synthesis and their function.

These patients are more prone to develop infections and gangrene that ultimately ends in amputation of the affected limb.

Conventional therapies fail to treat these ulcers.

These aspects lead to the development of eletrophysical modalities like pulsed electromagnetic fields to treat diabetic ulcers.

It is suggested that PEMF acts at cellular level, induce electrical currents in the melieu of wound fluid and influence various steps in the healing and hasten it.

It targets at various growth factors such as vascular endothelial growth factor which play a role in the new blood vessel formation that play an essential role in wound healing.

Thus pulsed electromagnetic field therapy is a non invasive, safe and effective method of adjunct treatment for diabetic foot ulcers.

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ANNEXURES

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg No.ECR/270/Inst./TN/2013
Telephone No. 044 25305301
Fax : 011 25363970

CERTIFICATE OF APPROVAL

To
Dr. Amareswari V.H.
Postgraduate M.D.(Physiology),
Madras Medical College,
Chennai – 600 003.

Dear Dr.Amareswari,

The Institutional Ethics Committee has considered your request and approved your study titled **"Evaluation of effect of Pulsed Electro Magnetic Field Therapy in patients with chronic non healing diabetic foot ulcers". No.01102014.**

The following members of Ethics Committee were present in the meeting held on 07.10.2014 conducted at Madras Medical College, Chennai-3.

1.	Dr.C.Rajendran, M.D.,	: Chairperson
2.	Dr.R.Vimala, M.D., Dean, MMC, Ch-3	: Deputy Chairperson
3.	Prof.R.Kalaiselvi, M.D., Vice-Principal, MMC, Ch-3	: Member Secretary
4.	Prof.R.Nandhini, M.D., Inst.of Pharmacology, MMC	: Member
5.	Prof.K.Ramadevi, Director i/c, Inst.of Biochemistry, MMC	: Member
6.	Prof.Saraswathy, M.D., Director, Pathology, MMC, Ch-3	: Member
7.	Prof.S.G.Sivachidambaram, M.D., Director i/c, Inst.of Internal Medicine, MMC	: Member
8.	Thiru S.Rameshkumar, Administrative Officer	: Lay Person
9.	Thiru S.Govindasamy, B.A., B.L.,	: Lawyer
10.	Tmt.Arnold Saulina, M.A., MSW.,	: Social Scientist

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/ informed consent and asks to be provided a copy of the final report.

Member Secretary, Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
Chennai-600 003

INFORMED CONSENT FORM

Title of the study"Evaluation of the effect of Pulsed Electro Magnetic Field Therapy in Chronic non healing Diabetic foot ulcers"
Name of the Participant:
Name of the Principal Investigator: Dr. AMARESWARI V.H.
Name of the Institution:

Institute of Physiology and Experimental Medicine,
Madras Medical College and Govt. General Hospital,
Chennai - 3

Documentation of the informed consent

I _____ have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I am over 18 years of age and, exercising my free power of choice, hereby give my consent to be included as a participant in

"**Evaluation of the effect of Pulsed Electro Magnetic Field Therapy in chronic non healing diabetic foot ulcers"**

1. I have read and understood this consent form and the information provided to me.
2. I have had the consent document explained to me.
3. I have been explained about the nature of the study.
4. I have been explained about my rights and responsibilities by the investigator.
5. I have been informed the investigator of all the treatments I am taking or have taken in the past _____ months including any native (alternative) treatment.
6. I have been advised about the risks associated with my participation in this study.
7. I agree to cooperate with the investigator and I will inform him/her immediately if I suffer unusual symptoms.
8. I have not participated in any research study within the past_____month(s).
9. I am aware of the fact that I can opt out of the study at any time without having to give any reason and this will not affect my future treatment in this hospital.
10. I am also aware that the investigator may terminate my participation in the study at any time, for any reason, without my consent.
12. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors,

regulatory authorities, Govt. agencies, and IEC. I understand that they are publicly presented.
13. I have understood that my identity will be kept confidential if my data are publicly presented.
14. I have had my questions answered to my satisfaction.
15. I have decided to be in the research study.
I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form I attest that the information given in this document has been clearly explained to mean and understood by me, I will be given a copy of this consent document.

For adult participants:

Name and signature / thumb impression of the participant (or legal representative if participant incompetent)

Name _____ Signature_____

Date_____

Name and Signature of impartial witness (required for illiterate patients):

Name _____ Signature_____

Date_____

Address and contact number of the impartial witness:

Name and Signature of the investigator or his representative obtaining consent:

Name _____ Signature_____

Date_____

ஆராய்ச்சி ஒப்புதல் படிவம்

ஆராய்ச்சி தலைப்பு

சர்க்கரை நோயினால் ஏற்படும் கால் ஆராப்புண்கள் காந்த சக்தியின் விளைவுகளைப் பற்றிய ஆராய்ச்சி.

பெயர்	:	தேதி	:
வயது	:	உள் நோயாளி எண்	:
பால்	:	ஆராய்ச்சி சேர்க்கை எண்	:

இந்த ஆராய்ச்சியின் விவரங்களும் அதன் நோக்கமும் முழுமையாக எனக்கு தெளிவாக விளக்கப்பட்டது.

எனக்கு விளக்கப்பட்ட விஷயங்களை புரிந்துகொண்டு நான் எனது சம்மதத்தை தெரிவிக்கிறேன்.

இந்த ஆராய்ச்சியில் யாருடைய நிர்பந்தமின்றி என் சொந்த விருப்பத்தின்பேரில் நான் பங்கு பெறுகின்றேன்.

இந்த ஆராய்ச்சியில் இருந்து நான் எந்நேரமும் விலகிக்கொள்ளலாம் என்பதையும் அதனால் எந்த பாதிப்பும் ஏற்படாது என்பதையும் நான் புரிந்துகொண்டேன்.

சர்க்கரை நோயினால் ஏற்படும் கால் ஆராப்புண்கள் காந்த சக்தியின் விளைகள் பற்றிய இந்த ஆராய்ச்சின் விவரங்களை கொண்ட தகவல்களை பெற்றுக்கொண்டேன்.

நான் என்னுடைய சுய நினைவுடனும் மற்றும் முழு சுதந்திரத்துடனும் இந்த மருத்துவ ஆராய்ச்சியில் என்னை சேர்த்துக்கொள்ள சம்மதிக்கிறேன்.

ஆராய்ச்சியாளர் கையொப்பம் **பங்கேற்பாளர் கையொப்பம்**

நாள் :
இடம் :

PROFORMA

1. Name:

2. Age:

3. Sex:

4. Address

5. H/o Smoking/Alcohol/DM/HT

6. H/o Heart disease/Pace maker /PVD/Venous ulcers

7. Duration of diabetes:

8. Treatment for diabetes: On hypoglycemic drugs Yes/No

9. FBS:

10. PPBS:

11. Urea:

12. Creatinine:

13. BP:

14. Duration of ulcer:

15. Callus: Present (or) Absent

16. Sensory Loss (NDS) score:

17. Biothesiometer

18. Bounding Peripheral Pulses:

19. Ankle brachial index:

20. Bone to probe test:

21. Wagner's Grade: 1or 2

22. Ulcer site: (Right or Left), (Plantar or Heel or Toes or Leg)

23. Ulcer length in cm:

24. Ulcer width in cm:

25. Ulcer surface area in cm^2:

26. Tissue type: Necrotic tissue or Slough or Granulation tissue or Epithelial tissue or closed

27. Exudate amount: None or light or Moderate or Heavy

28. Serum VEGF levels:

MASTER CHART

CASE NO	AGE YRS	SEX	DURATION DM YRS	DURATION ULCER MONTHS	FBS mg/dl	PPBSmg/dl	SYS BP	DIA BP	CALLUS FOOT PRE	CALLUS FOOT POST	NDS PRE	NDS POST	BIOTHESIOMETER PRE	BIOTHESIOMETER POST	BOUNDING PULSES	ABI	PBT	ULCER SITE RT	ULCER SITE LT	WAGNER'S GRADE	LENGTH PRE cm	LENGTH POST cm	WIDTH PRE cm	WIDTH POST cm	SURFACE AREA PRE cm^2	SURFACE AREA POST cm^2	% REDUCTION SURFACE AREA	DEPTH pre cm	DEPTH post cm	TISSUE TYPE PRE	TISSUE TYPE POST	EXUDATE AMOUNT PRE	EXUDATE AMOUNT POST	VEGF pg/ml PRE	VEGF pg/ml POST
1	60	male	10	3	130	180	140	90	N	N	6	3	25	10	Y	1	N	1		2	4	1.5	2.5	1	10	1.5	85%	0.5	0.1	3	1	2	0	50	900
2	60	male	12	6	120	150	140	90	N	N	7	3	25	10	Y	0.9	N		1	2	2.5	2	1.8	1	4.5	2	56%	0.5	0.2	3	2	1	0	30	800
3	43	female	7	4	120	160	130	90	N	N	6	2	20	15	Y	1	N	1		1	0.5	0.2	0.5	0.5	0.25	0.1	60%	0.4	0.3	3	2	1	0	50	750
4	50	male	8	6	110	140	120	88	N	N	6	2	20	10	Y	0.9	N	2		2	4	2	1	0.5	4	1	75%	0.5	0.2	3	2	1	0	30	800
5	51	male	8	4	120	150	130	90	N	N	7	2	25	15	Y	1	N		1	2	3	1.5	1.5	0.8	4.5	1.2	73%	0.5	0.2	3	2	2	0	40	750
6	60	female	10	5	126	148	120	90	Y	N	6	2	20	10	Y	1	N	3		1	0.5	0.2	0.5	0	0.25	0	100%	0.5	0	3	0	1	0	10	1000
7	54	male	10	6	130	170	140	90	N	N	8	2	30	10	Y	0.9	N		2	1	3	2	1.5	0.8	4.5	1.60	64%	0.7	0.3	3	2	1	0	50	750
8	52	male	8	4	110	138	110	88	N	N	8	2	30	15	Y	0.9	N	1		2	3	1.2	2	1	6	1.2	80%	1	0.5	3	1	1	0	50	900
9	48	female	6	4	126	148	120	80	N	N	6	2	20	10	Y	1	N		4	2	2.5	1.2	1.8	1	4.5	1.2	73%	1	0.5	3	1	2	1	30	750
10	46	male	7	5	124	138	110	90	N	N	6	2	20	10	Y	1	N	2		1	3	2	1	0.5	3	1	67%	0.7	0.4	3	2	1	0	20	600
11	60	male	8	6	124	140	110	90	N	N	6	2	20	15	Y	1	N	2		1	3	1.5	1.5	1	4.5	1.5	67%	0.7	0.4	3	2	2	0	30	750
12	60	male	7	8	120	138	140	80	N	N	7	2	25	15	Y	1	N		1	2	4	2	2.5	1.5	10	3	70%	1	0.3	3	2	2	0	20	750
13	52	male	6	3	130	170	130	80	N	N	7	2	25	10	Y	1	N	4		1	2.8	1.5	2	1.2	5.6	1.8	68%	0.5	1	3	2	2	0	30	625
14	50	male	8	4	120	180	130	80	N	N	7	2	25	10	Y	1	N		2	1	2.7	1.7	2	1.3	5.4	2.21	59%	0.6	0.3	3	2	2	1	20	375
15	54	male	8	6	110	130	140	90	Y	N	7	2	25	15	Y	0.9	N	3		2	3	2.5	1.5	1.2	4.5	3	33%	1	0.8	3	3	1	1	40	70
16	60	male	8	3	130	130	130	80	N	N	7	2	25	15	Y	0.9	N		1	1	3	2	1.5	1	4.5	2	56%	0.5	0.2	3	2	1	0	30	375
17	60	male	9	6	120	138	140	80	Y	Y	8	2	30	15	Y	0.9	N		1	1	2.5	2	2	1	5	2	60%	0.5	0.2	3	2	2	0	50	650
18	58	male	10	6	120	150	120	80	Y	Y	8	2	30	10	Y	1	N	4		2	4	1.5	2.5	1.5	10	2.3	77%	0.8	0.4	3	2	1	0	50	750
19	52	male	7	4	120	160	130	80	Y	N	8	1	25	10	Y	0.9	N		1	1	0.7	0.2	0.8	0	0.56	0	100%	0.4	0	3	2	1	0	30	850
20	55	male	5	6	110	130	130	80	N	N	8	1	25	15	Y	1	N	1		1	0.5	0.4	0.5	0.3	0.25	0.12	52%	0.4	0.2	3	2	1	1	20	100
21	60	female	8	4	120	160	140	90	N	N	7	3	25	10	Y	0.9	N		2	2	2.5	2	1.7	1	4.25	2	53%	0.8	0.4	3	1	2	0	30	575
22	55	male	8	6	110	148	130	80	Y	N	9	3	30	15	Y	1	N	2		2	4	3.5	1	0.8	4	2.8	28%	0.8	0.6	3	3	2	1	20	80
23	60	female	7	6	110	138	130	80	N	N	9	4	30	10	Y	0.9	N		1	1	1	0.5	1	0.5	1	0.25	75%	0.6	0.3	3	1	2	0	10	600
24	48	male	7	5	120	150	120	90	N	N	7	2	25	15	Y	0.9	N	1		1	4	2.5	2.5	1.5	10	3.75	63%	0.6	0.3	3	2	1	0	30	575
25	58	male	6	4	110	130	130	90	N	N	8	2	25	10	Y	0.9	N		1	2	2.5	1.5	1.5	0.9	3.75	3.75	64%	1	0.4	3	2	1	0	20	700
26	52	male	7	4	110	140	140	80	N	N	8	2	25	15	Y	1	N	2		2	2.5	1.2	1.5	1	3.75	1.2	62%	0.8	0.3	3	1	1	0	50	625
27	58	male	6	4	106	150	120	90	N	N	8	2	25	10	Y	0.9	N		2	1	1	0.5	1	0.5	1	0.25	75%	0.8	0.5	3	2	1	0	50	900
28	56	female	7	4	110	140	130	80	N	N	8	2	25	10	Y	1	N	2		1	1	0.8	1	0.5	1	1	60%	0.5	0.2	3	1	1	0	40	625
29	60	female	8	5	120	150	130	80	N	N	8	2	25	15	Y	1	N		1	1	1.5	0.8	1	0.6	1.5	0.48	68%	0.6	0.2	3	2	1	0	30	750
30	60	male	8	6	110	138	120	80	N	N	8	4	25	10	Y	1	N	2		2	3.5	2.5	1.2	0.8	4.2	2	52%	0.8	0.4	3	2	2	0	30	450